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Origins and Effects of Extracellular α-synuclein: Implications in Parkinson's Disease

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Abstract Misfolding and abnormal aggregation of the neuronal protein α-synuclein has been implicated in the pathogenesis of Parkinson's disease and related neurological disorders, such as dementia with Lewy bodies. α-synuclein is a conventional cytosolic protein and is thought to exert its pathogenic function exclusively in the neuronal cytoplasm in a cell-autonomous manner. However, the current model is being challenged by a series of new observations that demonstrate the presence of α-synuclein and its aggregated forms in the extracellular fluid both in vivo and in vitro. Extracellular α-synuclein appears to be delivered by unconventional exocytosis of intravesicular α-synuclein, although the exact mechanism has not been characterized. Compared to the cytosolic α-synuclein, intravesicular α-synuclein is prone to aggregation and the potential source of extracellular aggregates. A number of tissue culture studies suggest that exposure to extracellular α-synuclein aggregates induces microglial activation, release of pro-inflammatory cytokines from astrocytes, and neurotoxicity. Thus, exocytosis of α-synuclein may be an important mechanism for amplifying and spreading degenerative changes from a small group of cells to its surrounding tissues, and it potentially provides therapeutic targets for halting the progression of the disease.

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Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disease whose primary clinical features include motor abnormalities, such as resting tremor, bradykinesia and rigidity (Fahn and Sulzer 2004). Pathologically, PD is characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta and the presence of inclusion bodies, called Lewy bodies and Lewy neurites, in the surviving neurons of the same region (Forno 1996). Although it is generally accepted that the loss of midbrain dopaminergic neurons is largely responsible for the major motor symptoms, this is not the only region showing pathologic changes in PD patients. Careful temporal staging of the pathological changes in PD patients has demonstrated that the Lewy pathology and cell loss first appear in lower brain stem nuclei, progressively ascend to the midbrain and finally to cortical areas in a highly predictable manner (Braak et al. 2004). As the disease progresses, the pathologic changes appear to spread in a defined pattern in the patient's brain. Progression of the Lewy pathology to the various regions outside the midbrain may account for the abundance of the secondary symptoms commonly observed in PD patients, such as depression, dementia, and various autonomic and sensory dysfunctions.

Although the cause of PD remains elusive, there is a large body of evidence suggesting that misfolding and abnormal aggregation of α-synuclein is an important component of the disease pathogenesis. Genetic linkage analyses have identified three missense mutations in the inherited forms of parkinsonism (Kruger et al. 1998; Polymeropoulos et al. 1997; Zarranz et al. 2004), and all the mutant variants have been shown to accelerate either oligomerization or fibrillation (Conway et al. 2000; Greenbaum et al. 2005). More recently, duplication or triplication of the locus containing the α-synuclein gene, resulting in increased expression of the protein, has been linked to familial PD (Chartier-Harlin et al. 2004; Ibanez et al. 2004; Singleton et al. 2003). This suggests that accumulation of wild type α-synuclein is sufficient to cause the disease. Importantly, fibrillar aggregates of α-synuclein seem to be the main component of Lewy bodies and Lewy neurites, and these are now considered the most reliable PD marker for postmortem diagnosis (Spillantini et al. 1998). Thus, aggregation of α-synuclein is also associated with the classical PD. In animal models, transgenic or viral vector-mediated overexpression of α-synuclein induced neuronal loss and Lewy body-like inclusion formation (Maries et al. 2003), further supporting the importance of α-synuclein in the neurodegenerative processes.

α-Synuclein is a classical cytosolic protein, and therefore it has been assumed that the pathogenic changes induced by the protein occur in the cytoplasm, thereby limiting the effect to the single cell. However, recent studies of extracellular α-synuclein suggest that the scope of pathogenic action goes beyond the cytoplasm of its origin. Here, I will review the recent data on the generation and effects of extracellular α-synuclein, and discuss the implications in pathogenesis of PD.

α-Synuclein in Human Body Fluid

In an attempt to find a biomarker for PD, extracellular α-synuclein was first detected in a small number of cerebrospinal fluid (CSF) samples from both PD and normal subjects (Borghi et al. 2000). Later, in a more controlled, careful study, El-Agnaf et al. (2003) demonstrated the presence of α-synuclein in human CSF and blood plasma in low nanomolar concentrations. Both studies used immunoprecipitation and Western blotting for the detection of α-synuclein and found no significant difference in the amount of α-synuclein in PD and control subjects. Two studies have addressed whether extracellular α-synuclein can be used as a diagnostic marker for PD, using a more sensitive and accurate ELISA method. One study measured the levels of plasma α-synuclein levels in 105 patients with PD, 38 patients with multiple system atrophy (MSA), and 51 age-matched controls, and found significant elevations in both patient groups (Lee et al. 2006). The other study measured the concentration of α-synuclein in CSF samples from 33 patients with PD and 38 controls, and showed that the levels were significantly lower in PD patients (Tokuda et al. 2006). In addition, El-Agnaf and colleagues (El-Agnaf et al. 2006) developed a novel ELISA specific for the oligomeric forms of α-synuclein and measured the levels of soluble α-synuclein aggregates in normal plasma and postmortem CSF from PD and control patients. They reported significantly elevated levels of oligomeric α-synuclein in 52% of the PD patients compared with only 14.8% in the control. However, due to a considerable overlap between the two groups, in the amounts of total α-synuclein and oligomeric forms, it remains unclear whether body fluid levels of α-synuclein is a realistic measure for a decisive diagnostic tool. However, these studies suggest that changes in the levels of extracellular α-synuclein are associated with the disease, and may provide new insights into the disease.

Secretion of α-synuclein

What is the origin of the extracellular α-synuclein in body fluids? How can a protein translated in the cytosol be present in the extracellular space, even in healthy individuals? The answers to these questions have begun to emerge from tissue culture studies demonstrating α-synuclein secretion from neuronal cells. Secretion of α-synuclein was independent of the expression method and was observed using a stable overexpressor (El-Agnaf et al. 2003), by transient transfection (Sung et al. 2005) and using viral vector-mediated expression (Lee et al. 2005). The latter study demonstrated that the release of α-synuclein from cells is not due to membrane leakage in dying cells by showing the lack of secretion of unrelated small cytosolic proteins (Lee et al. 2005). The secretion of endogenous α-synuclein from rat embryonic cortical neurons was demonstrated in the same study. Although the contribution from dying cells cannot be ruled out entirely, the secretion from the normal cells seems to be the main source of extracellular α-synuclein.

Mechanism of Secretion

What is the mechanism of α-synuclein secretion? Vesicle-mediated exocytosis appears to be the primary mechanism responsible for the secretion. The release of α-synuclein was inhibited at a low temperature (Lee et al. 2005), which blocks vesicle-mediated exocytosis. Since α-synuclein is a cytoplasmic protein, it must translocate across the vesicular

membranes before it can be exocytosed. It has indeed been demonstrated that a portion of cellular α-synuclein was present in the lumen of vesicles from rat brain homogenates, rat embryonic cortical neurons and human neuroblastoma cells (Lee et al. 2005). α-Synuclein can bind to phospholipid membranes in vitro. In live cells the membrane interaction seems to be highly dynamic (Fortin et al. 2005; Kim et al. 2006), and thus, it is possible that vesicle membrane-associated α-synuclein proteins do not remain bound to the vesicles during the vesicle isolation procedure. Instead, a large portion of α-synuclein protein that stably co-fractionates with vesicles from brain and cell homogenates, most likely represents the intravesicular form of the protein. Electron microscopy and density gradient ultracentrifugation suggested that the vesicles containing α-synuclein have morphologies and sedimentation properties similar to the dense core vesicles (Lee et al. 2005), but their exact identities remain unknown.

As predicted from the lack of an endoplasmic reticulum (ER) targeting signal peptide, the secretion of α-synuclein is insensitive to brefeldin A (Lee et al. 2005), suggesting that it is secreted via the ER/Golgi-independent, unconventional secretory pathway. Recent years have witnessed a large increase in the number of cytosolic proteins that are exported to the extracellular space (Fevrier and Raposo 2004; Nickel 2003). These proteins include cytokines and growth factors (e.g., interleukin-1α and -1β, fibroblast growth factor 1 and 2), as well as some classical cytosolic proteins (e.g., galectins, thioredoxin, heat shock proteins, cytoskeletal proteins, etc.). The mechanism of the unconventional secretion is not well understood. From what we know so far, there seem to be multiple mechanisms, some involving vesicular exocytosis (Nickel 2003). Given the increasing number of examples of unconventional secretion, it is becoming clear that secretion of cytoplasmic proteins via these pathways is much more common than initially believed.

Only a small portion of cellular α-synuclein is translocated into the vesicles and secreted and therefore, the vesicle entry must be a selective process (Fig. 1). How this selection process occurs is unknown. One possibility is that defective proteins are selected and translocated into vesicles and subsequently discarded from cells by exocytosis. If this hypothesis is correct, vesicular α-synuclein would be highly prone to aggregation. There is some evidence suggesting that this is the case. When the aggregation potential of α-synuclein isolated from brain vesicles and cytosolic preparations, as well as from vesicle and the cytosolic fractions of intact neuroblastoma cells, was compared, intravesicular α-synuclein formed aggregates at a much higher rate than the cytosolic protein (Lee et al. 2005). The aggregates can also be secreted and the secretion is inhibited by the low temperature block (Lee et al. 2005), suggesting a dependence on exocytosis. Furthermore, the secretion of α-synuclein and its aggregates was increased when the cells were subjected to stresses that cause accumulation of damaged proteins (e.g., proteasomal and mitochondrial inhibition) (Lee et al. 2005). Based on these results, it is tempting to speculate that only α-synuclein proteins with conformational defects translocate into vesicles, form aggregates and are secreted to the extracellular space. However, there are other potential mechanisms that should be considered. For example, specific post-translational modifications may play roles in the selection process. Characterization of the intravesicular and secreted α-synuclein proteins, as well as analysis of the secretory pathway, will help determine the mechanism of vesicular translocation and secretion of α-synuclein.

Intravesicular α-synuclein is more prone to aggregation than the cytosolic protein and this could be explained by other factors than protein conformation. The microenvironment of the vesicular lumen is quite different from the cytoplasm, which may alter the physicochemical properties of the protein and affect the kinetics of aggregation. Some of the lumenal characteristics and components of certain types of vesicles are known to accelerate the aggregation of α-synuclein. These include high calcium concentration (Lowe et al. 2004), low pH (Hoyer et al. 2002) and presence of glycosaminoglycans such as heparin (Cohlberg et al. 2002). Determination of the vesicular identity and the detailed biochemical properties of the vesicular α-synuclein are likely to provide insights into the mechanisms of vesicle translocation and accelerated aggregation.

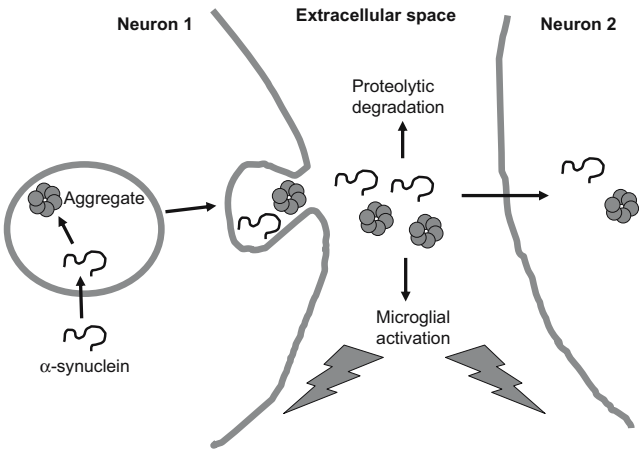

Figure 1 A hypothetical model for the function of extracellular α-synuclein. A small portion of the cytoplasmic α-synuclein is translocated into vesicles. The vesicle type/identity remains unknown. Intravesicular α-synuclein appears to be more prone to aggregation than the cytoplasmic form and both monomers and aggregates are secreted via exocytosis. Secreted α-synuclein may be removed from the extracellular space by proteolytic degradation and internalization into neighboring cells. Accumulation of α-synuclein in the extracellular space can activate microglia, causing neuroinflammation-mediated neurodegeneration

Effects of Extracellular α-synuclein

The role of secreted α-synuclein in the extracellular space can be inferred from studies using tissue culture systems. Several studies reported cytotoxic effects of extracellular α-synuclein and its internal hydrophobic fragment (non-amyloid component or NAC) when the proteins were added to the culture medium (Albani et al. 2004; Bodles et al. 2000; Du et al. 2003; El-Agnaf et al. 1998; Forloni et al. 2000; Lee et al. 2004; Seo et al. 2002; Sung et al. 2001). Some studies have demonstrated the toxic effect of fibrillar aggregates (Bodles et al. 2000; El-Agnaf et al. 1998), while other studies identified protofibrillar or oligomeric aggregates as the toxic culprit (Du et al. 2003). There are not many well-structured models for the mechanisms of toxicity. One model proposes that oligomeric α-synuclein can form annular structures with a central pore (Volles and Lansbury 2003). These aggregates can bind to membranes (Volles et al. 2001) and their membrane permeabilizing action has been demonstrated in synthetic model membranes, such as phospholipid liposomes (Volles et al. 2001) and planar bilayer membranes (Kayed et al. 2004). Insertion of these aggregates into the cell membrane would have a catastrophic effect on cell viability due to the free exchange of ions and small metabolites between the cytoplasm and the extracellular space. Although this toxic pore model explains explicitly the cytotoxicity of at least some oligomeric aggregates, the pores and the pore activity have yet to be demonstrated with α-synuclein proteins expressed in biological systems.

Another potential mechanism of neurotoxicity of extracellular α-synuclein, and especially its aggregate forms, may involve neuroinflammatory responses. This idea was tested in a recent study, where the effects of "aged" α-synuclein on microglial activation were examined. Addition of α-synuclein to the cell culture medium of a microglia–enriched culture produced classical signs of activated microglia, such as production of superoxide anions, intracellular reactive oxygen species and prostaglandin E2 (Zhang et al. 2005). Importantly, this α-synuclein-induced microglial activation led to dopaminergic neurotoxicity in a midbrain neuron/microglia mixed cell culture (Zhang et al. 2005). However, the α-synuclein treatment did not produce the neurotoxic effect in the absence of microglia. This study suggests that the interaction between α-synuclein and microglia could activate these cells, which in turn induces neurotoxicity. In a different study, it was shown that exposure to α-synuclein stimulated production of the pro-inflammatory factors, intercellular adhesion molecule-1 and interleukin-6, from human astrocytes and human U-373MG astrocytoma cells (Klegeris et al. 2006). Reactive astrocytes and activated microglia are common pathological features of PD, and of drug-induced and genetic animal models of PD. Considering the effects of recombinant α-synuclein proteins on glial cells, the secreted, extracellular forms of α-synuclein may also activate these cells and induce chronic inflammation.

Regardless of the mechanism of toxicity, if the extracellular α-synuclein contributes to the pathogenic process of PD, blocking its action might prevent the progression of the disease. It is noteworthy that α-synuclein immunization has beneficial effects, at least in a transgenic mouse model of PD that overexpresses human α-synuclein (Masliah et al. 2005). For immunization, the transgenic animals were vaccinated with human α-synuclein. The mice produced high affinity antibodies and showed a reduction in α-synuclein aggregation and neurodegeneration (Masliah et al. 2005). The underlying mechanisms of the protective effects of α-synuclein immunization are unknown, but the target could be extracellular α-synuclein, which would be captured and potentially cleared by specific interactions with circulating antibodies.

Clearance of Extracellular α-synuclein

There are two potential mechanisms by which extracellular α-synuclein might be removed. The first mechanism relies on degradation by extracellular proteolytic enzymes. It has been shown that matrix metalloproteases can cleave both recombinant α-synuclein and α-synuclein secreted from human neuroblastoma cells (Sung et al. 2005). However, the proteolytic fragments can facilitate aggregation of α-synuclein and the resulting aggregates show higher toxicity (Sung et al. 2005). Thus, it is not clear whether the matrix metalloprotease-mediated cleavage of extracellular α-synuclein is a mechanism for removing the protein, or if it enhances the debilitating effects of the protein by producing more harmful species. The second potential mechanism for clearing extracellular α-synuclein involves uptake of the protein by neighboring cells, perhaps including the cells that secrete the protein. It has been shown that both neuronal cells and microglia can take up extracellular α-synuclein, at least in cell culture (Ahn et al. 2006; Sung et al. 2001; Zhang et al. 2005). The mechanism of uptake is controversial; one study showed a Rab5A-dependent internalization (Sung et al. 2001), implicating endocytosis, another study provided evidence for non-endocytic internalization (Ahn et al. 2006). We have recently found that monomeric α-synuclein can be translocated into cells without endocytosis, whereas aggregated forms enter cells via endocytosis (H.-J. Lee and S.-J. Lee, unpublished data). This suggests that different forms of α-synuclein use different mechanisms for cell entry. It is not known whether the internalized α-synuclein is degraded in the cells, and if so, by what mechanism this occurs.

Extracellular α-synuclein and the Progression of PD

PD is a progressive disease with pathological changes beginning and advancing in a highly predictable sequence through defined brain areas (Braak et al. 2004). Given this strikingly consistent pattern of pathologic progression, it does not seem far-fetched to consider the possibility of progressive propagation of pathogenic events through the affected areas. Propagation of pathogenic events by exogenous pathogens has been well documented in prion disorders. In these disorders, pathogenic prion aggregates can "infect" cells and nucleate the polymerization of normal cellular prion proteins (Caughey 2000). A similar mechanism of pathogenic propagation may operate in Alzheimer's disease. Administration of brain homogenates prepared from Alzheimer's patients or a transgenic mouse model of the disease caused earlier and more severe plaque pathology and neurodegeneration (Meyer-Luehmann et al. 2006). Although no such experiments have been performed in models of PD, secretion of α-synuclein and its aggregates into the extracellular space and the subsequent uptake of these proteins by adjacent neurons certainly satisfy the basic requirement for the propagation of pathogenic events. This idea should be testable using the transgenic animal model expressing human α-synuclein.

Future Perspectives

Despite the rapid expansion of research focused on α-synuclein, its precise role in PD pathogenesis has yet to be defined. Furthermore, we do not fully understand the normal behavior and function of this protein, and perhaps more importantly, we have yet to determine what aspect of its normal biology is relevant to the pathogenic transition of the protein. Recent studies have documented properties of α-synuclein that are atypical for a cytosolic protein, these include intravesicular localization and exocytic secretion. The presence of α-synuclein in human CSF and blood plasma provides in vivo validation of these properties. Furthermore, aggregated α-synuclein can be secreted by exocytosis in cell culture models, and the body fluids from PD patients contain elevated levels of oligomeric α-synuclein when compared with age-matched controls. There are several outstanding questions concerning the secretion of α-synuclein and the effects of the extracellular protein. What are the mechanisms of selection and translocation of α-synuclein into vesicles? What is the identity of the vesicles that contain α-synuclein? Do α-synuclein proteins induce neuroinflammatory responses after being secreted into the extracellular space? Which α-synuclein species have bioactivity in the extracellular space? How is extracellular α-synuclein removed? Does extracellular α-synuclein play a role in the progression of PD? Some of these questions are currently being investigated, testing specific hypotheses, while others await further characterization of the basic mechanisms before they can be addressed. I anticipate that we will see an increasing number of experiments carried out in this field and that many of these questions will be answered in the near future. This progress will increase our understanding of the basic biology of α-synuclein and potentially provide insights that will lead to novel strategies for effective PD diagnosis and therapy.

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References

Ahn, K. J., Paik, S. R., Chung, K. C., & Kim, J. (2006). Amino acid sequence motifs and mechanistic features of the membrane translocation of alpha-synuclein. *Journal of Neurochemistry, 97*, 265–279.

Albani, D., Peverelli, E., Rametta, R., Batelli, S., Veschini, L., Negro, A., et al. (2004). Protective effect of TAT-delivered alpha-synuclein: Relevance of the C-terminal domain and involvement of HSP70. *FASEB Journal, 18*, 1713–1715.

Bodles, A. M., Guthrie, D. J., Harriott, P., Campbell, P., & Irvine, G. B. (2000). Toxicity of non-Abeta component of Alzheimer's disease amyloid, and N-terminal fragments thereof, correlates to formation of beta-sheet structure and fibrils. *European Journal of Biochemistry, 267*, 2186–2194.

Borghi, R., Marchese, R., Negro, A., Marinelli, L., Forloni, G., Zaccheo, D., et al. (2000). Full length alpha-synuclein is present in cerebrospinal fluid from Parkinson's disease and normal subjects. *Neuroscience Letters, 287*, 65–67.

Braak, H., Ghebremedhin, E., Rub, U., Bratzke, H., & Del Tredici, K. (2004). Stages in the development of Parkinson's disease-related pathology. *Cell & Tissue Research, 318*, 121–134.

Caughey, B. (2000). Transmissible spongiform encephalopathies, amyloidoses and yeast prions: Common threads? *Nature Medicine, 6*, 751–754.

Chartier-Harlin, M. C., Kachergus, J., Roumier, C., Mouroux, V., Douay, X., Lincoln, S., et al. (2004). Alpha-synuclein locus duplication as a cause of familial Parkinson's disease. *Lancet, 364*, 1167–1169.

Cohlberg, J. A., Li, J., Uversky, V. N., & Fink, A. L. (2002). Heparin and other glycosaminoglycans stimulate the formation of amyloid fibrils from alpha-synuclein in vitro. *Biochemistry, 41*, 1502–1511.

Conway, K. A., Lee, S.-J., Rochet, J. C., Ding, T. T., Williamson, R. E., & Lansbury Jr., P. T. (2000). Acceleration of oligomerization, not fibrillization, is a shared property of both alpha-synuclein mutations linked to early-onset Parkinson's disease: Implications for pathogenesis and therapy. *Proceedings of the National Academy of Sciences of the United States of America, 97*, 571–576.

Du, H. N., Tang, L., Luo, X. Y., Li, H. T., Hu, J., Zhou, J. W., et al. (2003). A peptide motif consisting of glycine, alanine, and valine is required for the fibrillization and cytotoxicity of human alpha-synuclein. *Biochemistry, 42*, 8870–8878.

El-Agnaf, O. M., Jakes, R., Curran, M. D., Middleton, D., Ingenito, R., Bianchi, E., et al. (1998). Aggregates from mutant and wild-type alpha-synuclein proteins and NAC peptide induce apoptotic cell death in human neuroblastoma cells by formation of beta-sheet and amyloid-like filaments. *FEBS Letters, 440*, 71–75.

El-Agnaf, O. M., Salem, S. A., Paleologou, K. E., Cooper, L. J., Fullwood, N. J., Gibson, M. J., et al. (2003). Alpha-synuclein implicated in Parkinson's disease is present in extracellular biological fluids, including human plasma. *FASEB Journal, 17*, 1945–1947.

El-Agnaf, O. M., Salem, S. A., Paleologou, K. E., Curran, M. D., Gibson, M. J., Court, J. A., et al. (2006). Detection of oligomeric forms of alpha-synuclein protein in human plasma as a potential biomarker for Parkinson's disease. *FASEB Journal, 20*, 419–425.

Fahn, S., & Sulzer, D. (2004). Neurodegeneration and neuroprotection in Parkinson disease. *NeuroRx, 1*, 139–154.

Fevrier, B., & Raposo, G. (2004). Exosomes: Endosomal-derived vesicles shipping extracellular messages. *Current Opinion in Cell Biology, 16*, 415–421.

Forloni, G., Bertani, I., Calella, A. M., Thaler, F., & Invernizzi, R. (2000). Alpha-synuclein and Parkinson's disease: Selective neurodegenerative effect of alpha-synuclein fragment on dopaminergic neurons in vitro and in vivo. *Annals of Neurology, 47*, 632–640.

Forno, L. S. (1996). Neuropathology of Parkinson's disease. *Journal of Neuropathology and Experimental Neurology, 55*, 259–272.

Fortin, D. L., Nemani, V. M., Voglmaier, S. M., Anthony, M. D., Ryan, T. A., Edwards, R. H. (2005). Neural activity controls the synaptic accumulation of {alpha}-synuclein. *Journal of Neuroscience, 25*, 10913–10921.

Greenbaum, E. A., Graves, C. L., Mishizen-Eberz, A. J., Lupoli, M. A., Lynch, D. R., Englander, S. W., et al. (2005). The E46K mutation in alpha-synuclein increases amyloid fibril formation. *Journal of Biological Chemistry, 280*, 7800–7807.

Hoyer, W., Antony, T., Cherny, D., Heim, G., Jovin, T. M., & Subramaniam, V. (2002). Dependence of alpha-synuclein aggregate morphology on solution conditions. *Journal of Molecular Biology, 322*, 383–393.

Ibanez, P., Bonnet, A. M., Debarges, B., Lohmann, E., Tison, F., Pollak, P., et al. (2004). Causal relation between alpha-synuclein gene duplication and familial Parkinson's disease. *Lancet, 364*, 1169–1171.

Kayed, R., Sokolov, Y., Edmonds, B., McIntire, T. M., Milton, S. C., Hall, J. E., et al. (2004). Permeabilization of lipid bilayers is a common conformation-dependent activity of soluble amyloid oligomers in protein misfolding diseases. *Journal of Biological Chemistry, 279*, 46363–46366.

Kim, Y. S., Laurine, E., Woods, W., & Lee, S. J. (2006). A novel mechanism of interaction between alpha-synuclein and biological membranes. *Journal of Molecular Biology, 360*, 386–397.

Klegeris, A., Giasson, B. I., Zhang, H., Maguire, J., Pelech, S., & McGeer, P. L. (2006). Alpha-synuclein and its disease-causing mutants induce ICAM-1 and IL-6 in human astrocytes and astrocytoma cells. *FASEB Journal, 20*, 2000–2008.

Kruger, R., Kuhn, W., Muller, T., Woitalla, D., Graeber, M., Kosel, S., et al. (1998). Ala30Pro mutation in the gene encoding alpha-synuclein in Parkinson's disease. *Nature Genetics, 18*, 106–108.

Lee, E. N., Cho, H. J., Lee, C. H., Lee, D., Chung, K. C., & Paik, S. R. (2004). Phthalocyanine tetrasulfonates affect the amyloid formation and cytotoxicity of alpha-synuclein. *Biochemistry, 43*, 3704–3715.

Lee, H. J., Patel, S., & Lee, S. J. (2005). Intravesicular localization and exocytosis of alpha-synuclein and its aggregates. *Journal of Neuroscience, 25*, 6016–6024.

Lee, P. H., Lee, G., Park, H. J., Bang, O. Y., Joo, I. S., & Huh, K. (2006). The plasma alpha-synuclein levels in patients with Parkinson's disease and multiple system atrophy. *Journal of Neural Transmission, 113*, 1435–1439.

Lowe, R., Pountney, D. L., Jensen, P. H., Gai, W. P., & Voelcker, N. H. (2004). Calcium(II) selectively induces alpha-synuclein annular oligomers via interaction with the C-terminal domain. *Protein Science, 13*, 3245–3252.

Maries, E., Dass, B., Collier, T. J., Kordower, J. H., & Steece-Collier, K. (2003). The role of alpha-synuclein in Parkinson's disease: Insights from animal models. *Nature Reviews Neuroscience, 4*, 727–738.

Masliah, E., Rockenstein, E., Adame, A., Alford, M., Crews, L., Hashimoto, M., et al. (2005). Effects of alpha-synuclein immunization in a mouse model of Parkinson's disease. *Neuron, 46*, 857–868.

Meyer-Luehmann, M., Coomaraswamy, J., Bolmont, T., Kaeser, S., Schaefer, C., Kilger, E., et al. (2006). Exogenous induction of cerebral beta-amyloidogenesis is governed by agent and host. *Science, 313*, 1781–1784.

Nickel, W. (2003). The mystery of nonclassical protein secretion. A current view on cargo proteins and potential export routes. *European Journal of Biochemistry, 270*, 2109–2119.

Polymeropoulos, M. H., Lavedan, C., Leroy, E., Ide, S. E., Dehejia, A., Dutra, A., et al. (1997). Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science, 276*, 2045–2047.

Seo, J. H., Rah, J. C., Choi, S. H., Shin, J. K., Min, K., Kim, H. S., et al. (2002). Alpha-synuclein regulates neuronal survival via Bcl-2 family expression and PI3/Akt kinase pathway. *FASEB Journal, 16*, 1826–1828.

Singleton, A. B., Farrer, M., Johnson, J., Singleton, A., Hague, S., Kachergus, J., et al. (2003). alpha-Synuclein locus triplication causes Parkinson's disease. *Science, 302*, 841.

Spillantini, M. G., Crowther, R. A., Jakes, R., Hasegawa, M., Goedert, M. (1998). alpha-Synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with lewy bodies. *Proceedings of the National Academy of Sciences of the United States of America, 95*, 6469–6473.

Sung, J. Y., Kim, J., Paik, S. R., Park, J. H., Ahn, Y. S., & Chung, K. C. (2001). Induction of neuronal cell death by Rab5A-dependent endocytosis of alpha-synuclein. *Journal of Biological Chemistry, 276*, 27441–27448.

Sung, J. Y., Park, S. M., Lee, C. H., Um, J. W., Lee, H. J., Kim, J., et al. (2005). Proteolytic cleavage of extracellular secreted {alpha}-synuclein via matrix metalloproteinases. *Journal of Biological Chemistry, 280*, 25216–25224.

Tokuda, T., Salem, S. A., Allsop, D., Mizuno, T., Nakagawa, M., Qureshi, M. M., et al. (2006). Decreased alpha-synuclein in cerebrospinal fluid of aged individuals and subjects with Parkinson's disease. *Biochemical and Biophysical Research Communications, 349*, 162–166.

Volles, M. J., & Lansbury Jr., P. T. (2003). Zeroing in on the pathogenic form of alpha-synuclein and its mechanism of neurotoxicity in Parkinson's disease. *Biochemistry, 42*, 7871–7878.

Volles, M. J., Lee, S.-J., Rochet, J. C., Shtilerman, M. D., Ding, T. T., Kessler, J. C., et al. (2001). Vesicle permeabilization by protofibrillar alpha-synuclein: Implications for the pathogenesis and treatment of Parkinson's disease. *Biochemistry, 40*, 7812–7819.

Zarranz, J. J., Alegre, J., Gomez-Esteban, J. C., Lezcano, E., Ros, R., Ampuero, I., et al. (2004). The new mutation, E46K, of alpha-synuclein causes Parkinson and Lewy body dementia. *Annals of Neurology, 55*, 164–173.

Zhang, W., Wang, T., Pei, Z., Miller, D. S., Wu, X., Block, M. L., et al. (2005). Aggregated alpha-synuclein activates microglia: A process leading to disease progression in Parkinson's disease. *FASEB Journal, 19*, 533–542.