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REVIEW ARTICLE

# The Cell Biology of α-Synuclein

## *A Sticky Problem?*

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## Abstract

Parkinson's disease (PD) is the most common neurodegenerative motor disorder, marked by chronic progressive loss of neurons in the substantia nigra, thereby damaging purposeful control of movement. For decades, it was believed that PD was caused solely by environmental causes. However, the discovery of genetic factors involved in PD has revolutionized our attempts to understand the disease's pathology. PD now appears to be more polygenetic than previously thought and is most likely caused by a complex interaction of genetic risks and environmental exposures. The first gene found to be mutated in PD encodes for the presynaptic protein α-synuclein, which is also a major component of Lewy bodies and Lewy neurites, the neuropathological hallmarks of the disease. While these findings provide a classic example of how rare genetic mutations in disease can point to important pathways in idiopathic disease pathologies, much of the study of α-synuclein has focused on understanding how this protein undergoes the transition from an unfolded monomer to amorphous aggregates or Lewy body-like filaments rather than addressing what its fundamental function might be. Since alterations in synuclein function may predispose to the disease pathology of PD, regardless of the presence of genetic mutations, a more thorough understanding of the cellular regulation and function of α-synuclein may be of crucial importance to our understanding of this degenerating disorder.

**Index Entries:** Parkinson's disease; synuclein; synapse; lipids; oligomerization; degradation.

## Introduction

Over the last few years, the scientific approach to the study of Parkinson's disease (PD) has undergone a major transformation. Prior to that, Parkinson's disease was thought to be attributable solely to environmental causes. In 1998, Polymeropoulos and colleagues found that mutations in a small

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140 amino acid presynaptic protein, named α-synuclein, caused Parkinson's disease in families of Greco-Italian descent. α-Synuclein was first discovered in the electric organ of *Torpedo* (Maroteaux et al., 1988), and localized to the presynaptic nerve terminal and nuclear membrane. Subsequently, synuclein was found to be upregulated the brains of songbirds, where it appeared to function in the seasonal development of neurons that control song learning (George et al., 1995). It is now known that α-synuclein is a member of a family of small highly conserved proteins, including β- (Jakes et al., 1994) and γ-synuclein (Lavedan et al., 1998), as well as synoretin (Surguchov et al., 1999), that are encoded by different genes, and are enriched in the vertebrate nervous system. However to date, only α-synuclein has been implicated in PD. Adding to these discoveries was the finding that α-synuclein was a major component of Lewy bodies, the pathological hallmark in the brains of those with the disease (Spillantini et al., 1997). Thus, while mutations in α-synuclein are rare in PD, these findings have pointed researchers toward the study of a protein that presumably has a role in the pathogenesis of idiopathic PD, as well as in other Lewy body-containing diseases (Goedert, 2001). Based on this assumption, studies of α-synuclein have focused on its aggregation qualities, and animal models that may recapitulate the pathology of PD. However, many basic questions regarding the normal cell biology of this protein remain unanswered. This review is not intended as an exhaustive enumeration of the sum of literature on α-synuclein, but rather focuses on some important cell biological studies and what they may indicate about the function of this enigmatic protein.

## α-*Synuclein Expression and Localization*

Autosomal dominant PD is similar to other familial neurodegenerative diseases, such as Alzheimer's disease and frontotemporal dementia, in which the defective gene encodes or increases the expression of the major molecular constituent of the neuropathological lesion (*see* Hardy and Gwinn-Hardy, 1998; Goedert, 2001). In the case of PD, missense mutations in the α-synuclein gene cause inherited forms of the disease (Polymeropoulos et al., 1997; Kruger et al., 1998), and α-synuclein itself is a major component of the filamentous aggregates that accumulate in Lewy bodies in sporadic PD and other Lewy body-containing disorders (*see* Spillantini et al., 1997; Goedert, 2001). Unlike these other diseases, mutations that result in increased levels of expression of α-synuclein have yet to be identified. Recent studies have demonstrated, however, that a dinucleotide repeat polymorphism (referred to as NACP-Rep1) located ~10 kb upstream of the translational start of α-synuclein gene (Xia et al., 1996; Touchman et al., 2001), participates in the control of its expression (Touchman et al., 2001). Additionally, certain alleles of the NACP-Rep1 locus have been shown to be associated with increased risk of sporadic PD (Kruger et al., 1999; Tan et al., 2000; Farrer et al., 2001), suggesting that variable expression of the gene may play a role in the pathogenesis of the disease.

Transcription of α-synuclein appears to be developmentally regulated; in rodents, mRNA levels peak in the first few postnatal weeks of development and then decrease thereafter (Petersen et al., 1999). Protein levels of synuclein, however, increase during development and are sustained at fairly high levels throughout adulthood (Petersen et al., 1999), suggesting that its levels may be controlled by as yet undiscovered post-transcriptional mechanisms. Secondary structure analysis of the 5-prime and 3-prime regions of the α-synuclein mRNA may point to regulatory control regions that determine message stability, translatability, or targeting. In rodents (Withers et al., 1997; Hsu et al., 1998) and humans (Bayer et al., 1999; Galvin et al., 2001) α-synuclein protein redistributes from cell bodies to synaptic terminals in the early weeks of development in concert with other presynaptic proteins, and remains there in adults. This redistribution is likely via the association of synuclein with membranes targeted to developing synapses (Jensen et al., 1999). Although there are subtle differences, α-synuclein appears to be expressed ubiquitously throughout the brain (Lavedan, 1998), with synuclein mRNA levels actually lowest in the brain region most susceptible in PD, the substantia nigra (Rockenstein et al., 2001). Interestingly, decreased synuclein mRNA levels were observed in the substantia nigra from patients with PD (Neystat et al., 1999), raising the possibility that decreases in synuclein levels are an early change in the process leading to neuronal degeneration.

To date, no genetic modifiers of α-synuclein expression have been found; however, various cellular treatments have been shown to affect synu-

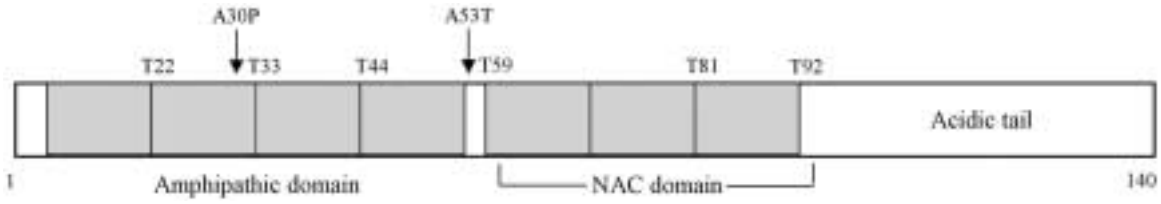


Fig. 1. Schematic organization of α-synuclein. The coding region of α-synuclein is 140 amino acids long and contains seven repeats (striped boxes) of a degenerate 11-residue consensus sequence, pKTKEGVaxaA, where p represents a polar residue, a represents an apolar residue, and x represents any residue. These amphipathic repeats likely form five alpha helices that resemble the class A2 family of apolipoproteins, with the indicated threonine residues in the center of the apolar face (Perrin et al., 2000). The two PD-linked mutations (A30P and A53T) are indicated. The NAC domain is the portion of α-synuclein identified from neuritic plaques in Alzheimer's disease (Ueda et al., 1993). Within this domain, repeat six is entirely hydrophobic and deviates from the consensus sequence of the other repeats (Clayton and George, 1998). Also within the NAC domain, α-synuclein contains eleven residues specifically absent in β-synuclein (amino acids 73–83).

clein levels, including nerve growth factor (NGF) (Stefanis et al., 2001), 1-methyl-4-phenyl-1,2,3,6-tetrahydropiridine (MPTP) (Vila et al., 2000), certain inflammatory cytokines and cellular stress (Tanji et al., 2001), and during megakaryocyte differentiation (Hashimoto et al., 1997). From these data, a clearer understanding of the transcriptional and translational regulation of synuclein expression is needed before we can understand how any changes in these mechanisms may affect the disease process.

## *The Hydrophobic Connection*

Purified α-synuclein shows little secondary structure in aqueous solution (Weinreb et al., 1996; Biere et al., 2000; Eliezer et al., 2001). The dominant feature of α-synuclein, however, is the presence of a series of amphipathic domains that span roughly the N-terminal two-thirds of the molecule (Fig. 1). Upon incubations with membranes, these domains adopt an extended alpha helical secondary structure similar to apolipoprotein class A2 molecules (Davidson et al., 1998; Eliezer et al., 2001) through which membrane binding becomes stabilized (Narayanan and Scarlata, 2001). These lipid-binding domains are conserved in all members of the synuclein family throughout all species examined. Partially included within this amphipathic region is a relatively hydrophobic stretch of amino acids, within α-synuclein's so-called NAC domain, that appears to distinguish α- from β-synuclein insofar as its capacity to form filaments in vitro and its selective inclusion within Lewy bodies (Biere et al., 2000; Giasson et al., 2001). The C-terminal third of α-synuclein is rich in acidic residues and likely remains free and unfolded upon membrane binding, thus providing a site for interactions with potential binding partners (Eliezer et al., 2001) (Fig. 2).

Although much work has demonstrated a preference for α-synuclein binding to synthetic membranes containing acidic phospholipids (Davidson et al., 1998; Jo et al., 2000), the specificity of its association with cellular membranes remains unclear. Undoubtedly, synuclein's synaptic localization indicates an affinity for synaptic membranes (Iwai et al., 1995; Withers et al., 1997; Murphy et al., 2000). However, immunoelectron microscopy (Clayton and George, 1999), as well as cell-fractionation studies (George et al., 1995; Irizarry et al., 1996; Kahle et al., 2000), suggests that synuclein is not stably associated with synaptic membranes. Although this is consistent with synuclein's structural similarity to the exchangeable apolipoproteins (George et al., 1995; Clayton and George, 1998), the actual mechanism that regulates synuclein's association with membranes is not known. Inasmuch as the C-terminal acidic tail of synuclein appears dispensable for membrane binding (Perrin et al., 2000), it is likely that post-translational modifications such as phosphorylation that can occur on Ser 129 (Okochi et al., 2000; Pronin et al., 2000) and/or Tyr 125 (Ellis et al., 2000; Nakamura et al., 2001), do not influence synuclein's ability to bind membranes directly (Narayanan and Scarlata, 2001). These modifications may, however, influence the kinetics of membrane binding or modulate synuclein's association

with various binding partners that then regulate membrane association. Determining the kinetics of synuclein's membrane on/off rate, therefore, in both purified and cellular systems is likely important for understanding not only its normal function, but in determining if alterations in this rate are affected by PD mutations that may influence synuclein's propensity for aggregation (*see* below). In this regard, the PD mutant A30P shows slightly reduced binding to synthetic membranes compared with wild-type synuclein (Perrin et al., 2000), and binds significantly less well to specific membrane compartments within intact cells (Cole et al., 2002). In contrast, the A53T mutation likely increases synuclein's affinity for membranes (Sharon et al., 2001; Cole et al., 2002). Since purified Lewy bodies contain lipid components (Gai et al., 2000), the possibility exists that if membrane binding is important in Lewy body formation, the different PD mutants cause disease by nonidentical mechanisms.

Based on in vitro binding experiments (Davidson et al., 1998; Perrin et al., 2000), it is likely that synuclein's association with synaptic membranes is regulated by the lipid composition of the membranes themselves. In vitro evidence suggests that synuclein may function as a negative regulator of the PIP2-dependent activation of phospholipase D2 (Jenco et al., 1998), and that phosphorylated forms of synuclein may modulate PLD2 activity (Pronin et al., 2000). Given the key role of phosphoinositides in a variety of synaptic processes (Osborne et al., 2001), synuclein may preferentially associate with or regulate the maintenance of ordered membrane microdomains and serve to regulate synaptic membrane signaling and/or morphogenesis. Although phosphorylation of α-synuclein has yet to be documented in brain, synuclein's ability to be phosphorylated by G-protein coupled receptor kinases (GRKs; (Pronin et al., 2000) and the Src family of nonreceptor tyrosine kinases (Ellis et al., 2000; Nakamura et al., 2001) in vitro and in culture systems support a role for synuclein in synaptic signaling pathways.

A more direct role for synuclein in modulating synaptic membrane morphogenesis is suggested by observations demonstrating a reduction in synaptic vesicle pools in the hippocampus of both antisense-treated cultured neurons (Murphy et al., 2000) and α-synuclein knockout mice (Cabin et al., manuscript submitted). In addition, synuclein has recently been proposed to act as a fatty acid binding protein (FABP) (Sharon et al., 2001). One function of the FABPs is to ferry free fatty acids between aqueous and membrane-bound compartments for a variety of purposes, including phospholipid synthesis, hydrolysis, and storage (*see* Storch and Thumser, 2000). Although structurally dissimilar to FABPs (Hertzel and Bernlohr, 2000), or other known nonspecific lipid transfer proteins (Choinowski et al., 2000), synuclein may undergo conformational changes upon fatty acid binding that allows it to transiently deliver lipids to various subcellular compartments. Functional analysis of fatty acid uptake and transport (e.g., conversion to phospholipids, triglycerides, etc.) needs to be performed in cell- or animal-based systems before synuclein's role as a bona-fide FABP can be concluded. Synuclein has also been proposed to function as a molecular chaperone that prevents denaturation of selected proteins (Kim et al., 2000; Souza et al., 2000b). This is likely to occur via synuclein's ability to bind to and stabilize hydrophobic domains that become exposed during protein denaturation. Given its well-known capacity to bind lipids, one possibility is that synuclein may act as a type of chaperone, not by binding to exposed hydrophobic domains in unfolded proteins, but by interacting with the hydrophobic domains of the membranes themselves. This 'lipid chaperone' function may normally serve to protect neuronal membranes from oxidative damage, since synaptic vesicles are enriched in phospholipids containing polyunsaturated acyl chains that are uniquely sensitive to lipid peroxidation (Deutsch and Kelly, 1981; Moore and Roberts, 1998; Refsgaard et al., 2000). By this mode of action, synuclein may also serve as a possible sink for reactive aldehyde groups generated by lipid peroxidation (Mattson, 1998) and may offer one explanation for the high relative abundance of synucleins in nervous tissue (Shibayama-Imazu et al., 1993; Iwai et al., 1995).

## *Synuclein Oligomerization*

Although the major component of Lewy bodies and Lewy neurites are 50–700 nm long filaments composed of α-synuclein (Arima et al., 1998; Spillantini et al., 1998), it remains unclear whether these insoluble aggregates are themselves the toxic species in PD, or whether oligomeric precursors (Hashimoto et al., 1998; Wood et al., 1999) or so-called protofilaments (*see* Goldberg and Lansbury, 2000) are most

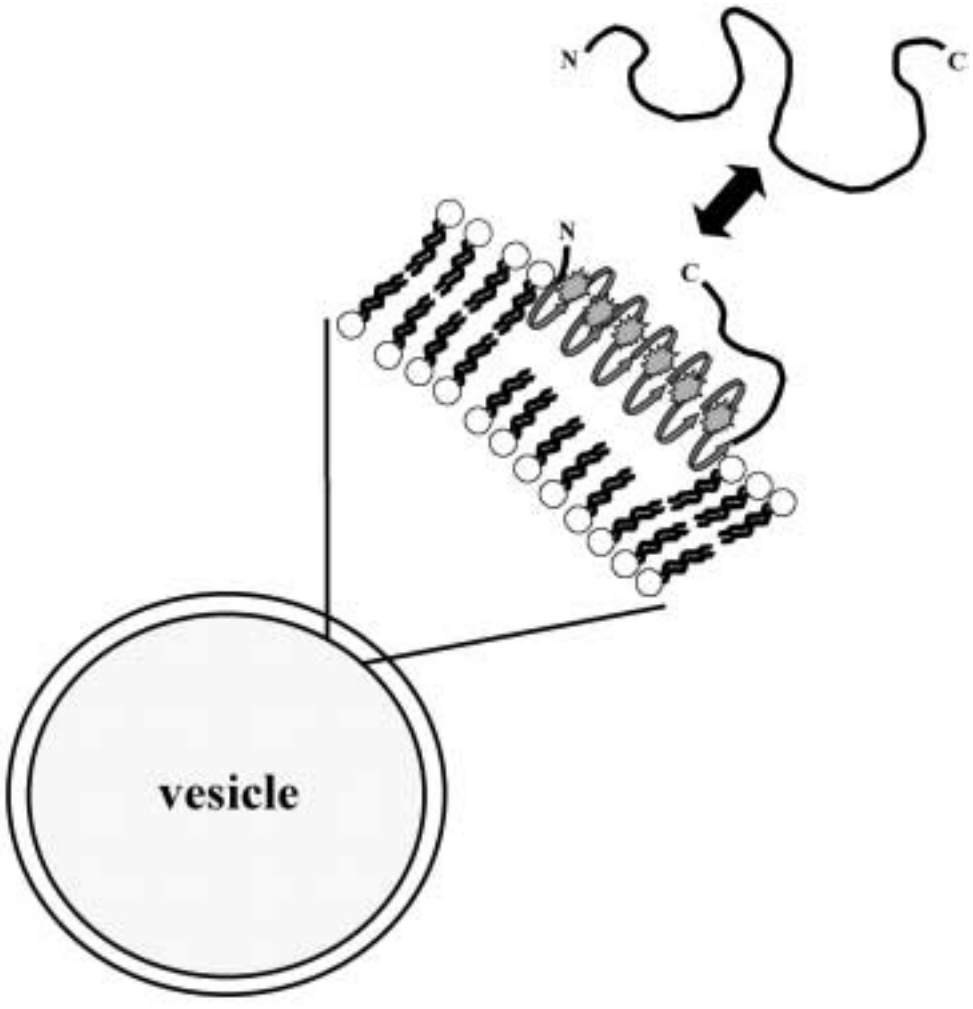


Fig. 2. Schematic representation of the transition of α-synuclein from a natively unfolded molecule in solution to one adopting an alpha-helical conformation in the presence of membranes. Shown is an expanded portion of the lipid bilayer from a synaptic vesicle. When associated with lipid, the nonpolar face of the amphipathic domain is buried deep into the hydrophobic interior of the lipid monolayer. The stars indicate the interfacial lysine residues that increase the lipid affinity of the amphipathic helices and help anchor the helix in the lipid bilayer (Mishra and Palgunachari, 1996). The acidic C-terminal tail remains free and unstructured (Eliezer et al., 2001). What determines synuclein's membrane on/off rate, and whether PD mutations affect the kinetics of this process are not known. Also unclear is the relationship between membrane binding and synuclein oligomer/filament formation (*see* text).

important in the establishment of disease. What is becoming clear, however, is that filament assembly is a nucleation-dependent process (Wood et al., 1999) and occurs via the amphipathic domains in the N-terminus of the protein (Crowther et al., 1998). In addition, this process appears to progress through partially folded intermediates that, at least in vitro, form under a wide variety of conditions (*see* Uversky et al., 2001a). Covalent stabilization and accumulation of these intermediates may then serve to nucleate subsequent fibril formation (Wood et al., 1999; Conway et al., 2000). The sizes of these intermediates likely include small oligomers, particularly dimers and trimers (Jensen et al., 1997; Baba et al., 1998; Hashimoto et al., 1998; Langston et al., 1998; Campbell et al., 2001), and their formation

likely results in partial conversion of synuclein to β-sheet rich structures (Volles et al., 2001). Descriptions of possible branched pathway intermediates that lead either to fibril formation or to amorphous aggregates (Uversky et al., 2001c) are still incomplete, however, as well as whether aggregation of PD mutants proceeds by identical pathways as wild-type synuclein.

Uversky and colleagues have illustrated that natively unfolded proteins are generally characterized by a combination of low overall hydrophobicity and large net charge. Thus, any alterations in a protein's environment leading to an increase in its hydrophobicity and/or decrease in its net charge are likely to lead to partially folded conformations (Uversky et al., 2000). Thus, it is not surprising that many of the molecules shown to induce conformational changes in synuclein affect these parameters. Clearly, membrane binding is the most significant; this results in the conversion of a natively unfolded molecule to one with a modular structure composed of alpha helices positioned within the N-terminal two-thirds of the molecule and an unstructured and exposed acidic C-terminus (Davidson et al., 1998; Jo et al., 2000; Eliezer et al., 2001). Additional alterations in synuclein structure have been shown to be directed primarily by nonpolar interactions and include its binding to fatty acids (Perrin et al., 2001; Sharon et al., 2001), hydrophobic pesticides (Uversky et al., 2001c) and dyes (Shin et al., 2000; Lee et al., 2001a), organic solvents (Kim et al., 2000), hydrophobic peptides (Paik et al., 1998), and exposed domains of partially denatured proteins (Kim et al., 2000; Souza et al., 2000b). Concurrently, decreases in synuclein's net charge are likely to result from exposure to metals that reduce electrostatic repulsion within synuclein's acidic C-terminus (Paik et al., 1999; Uversky et al., 2001b). The conformational intermediates that form under these conditions are in equilibrium with natively unfolded synuclein, and presumably any mechanism(s) that stabilizes these structures would lead to highly populated and potentially toxic intermediates. This likely includes the formation of cross-linked synuclein oligomers generated under oxidative stress conditions, such as the formation of dityrosine crosslinks (Souza et al., 2000a), and lipid peroxidation-generated adducts (Mattson, 1998; Perrin et al., 2001). Interestingly, within cell extracts synuclein oligomers appear to preferentially associate with membranes rather than in the cytosol (Lee et al., 2001c) (Cole et al., 2002), and thus may be formed via lipid peroxidation-based crosslinking of the numerous lysine residues positioned at the membrane/cytosol interface of membrane-bound synuclein (Clayton and George, 1998; Lee et al., 2001c; Perrin et al., 2001). It is therefore important to determine the role of membranes in Lewy body formation. Is the "seed" in Lewy body formation initiated by membrane-bound or cytosolic synuclein, and does synuclein oligomerization precede membrane binding, or are oligomers generated subsequent to the conformational alterations that occur in the synuclein monomer after binding to membranes?

One major unanswered question concerns the selective degeneration of dopaminergic neurons in the substantia nigra of patients with PD. Similar loss of neurons has been observed in brains of flies expressing wild-type and PD mutant synucleins (Feany and Bender, 2000) and in rats with chronic exposure to the pesticide rotenone (Betarbet et al., 2000). Much work has focused on the role of excessive oxidative stress generated by oxidation of the endogenous neurotransmitter dopamine, the formation of neuromelanin, and the presence of high concentrations of iron in the substantia nigra (Zhang et al., 2000a; Barzilai et al., 2001). Recently, Lansbury and colleagues demonstrated that synuclein fibrillization could be reduced and the proportion of synuclein oligomers/protofibrils increased in the presence of oxidized forms of dopamine and dopamine-related compounds (Conway et al., 2001). These compounds likely mediate the formation of synuclein-dopamine adducts and result in the formation of synuclein cross-linked oligomers incapable of progressing into synuclein fibers. Whether dopamine-based synuclein adducts are generated in vivo is unknown, as is the structural relationship between these synuclein oligomers/protofilaments and oligomers generated by other oxidative mechanisms (*see* above). Depending on whether synuclein filaments or oligomers/protofilaments are the toxic species in PD, it is unclear whether therapies based on targeted lowering of dopamine would be beneficial or detrimental.

### *Synuclein Binding Partners*

Aside from its inherent lipid binding properties, α-synuclein has been shown to interact with a number of proteins, through which their individ-

ual activities may be modulated. As mentioned earlier, α-synuclein (as well as β-synuclein) was shown to inhibit the activity of PLD2 in vitro (Jenco et al., 1998). Although direct binding of synuclein with PLD2 was not determined (Jenco et al., 1998), nor has an effect synuclein on PLD2 activity in cells or cell extracts been reported, this attractive observation fits with data for a potential role for synuclein in membrane trafficking events at the neuronal synapse. A direct interaction was observed between the NAC domain of α-synuclein and the C-terminal tail of the presynaptic human dopamine transporter (hDAT) (Lee et al., 2001b). Functionally, this interaction enhanced delivery of hDAT from internal membrane compartments to the cell surface and accelerated cellular dopamine uptake. Although synuclein did not colocalize with plasma membrane-associated hDAT as might be expected for a stable interaction, these results provide further evidence for a role for synuclein in membrane trafficking of synaptic membrane proteins. Whether localization of the DAT is altered in synuclein knockout or PD mutant mice is therefore an important question, as is the role of synuclein in dopamine neurotransmitter uptake and release (Abeliovich et al., 2000).

The first protein shown to physically bind to α-synuclein, termed synphilin-1, was revealed by a yeast two-hybrid study (Engelender et al., 1999). Synphilin-1 is a novel 90 kDa cytoplasmic protein of unknown function with several ankyrin-like repeats, and a coiled-coil domain; thus one proposed function of synphilin-1 is as an adaptor protein to anchor α-synuclein to intracellular proteins involved in vesicle transport and cytoskeletal function (Engelender et al., 1999). Synphilin-1 binds to synuclein primarily within synuclein's N-terminal amphipathic domain (amino acids 1-39) (Engelender et al., 1999). Whether synuclein is capable of binding simultaneously to both synphilin-1 and membranes is unknown. Coexpression of synphilin-1 with the NAC domain (amino acids 61–95) of α-synuclein, but not the full-length protein, induced the formation of phase dense eosinophilic cytoplasmic inclusions in a small percentage of cells (Engelender et al., 1999). How the NAC domain induced these synphilin-1 inclusions in the apparent absence of direct binding (Engelender et al., 1999) is not clear. Also somewhat contradictory are more recent findings from the same group that full-length synuclein was capable of forming cytoplasmic inclusions when coexpressed with synphilin-1 (Chung et al., 2001). Whether the formation of these inclusions is due to synphilin-1-induced structural changes in synuclein that predispose it to oligomerization/aggregation is not known (*see* above).

Recently, synphilin-1 was shown to bind and be ubiquitinated by the E3 ubiquitin-protein ligase, parkin (Chung et al., 2001). Mutations in parkin cause autosomal-recessive juvenile-onset PD (AR-JP) (Kitada et al., 1998), most likely by disrupting its E3 ubiquitin-protein ligase activity (Imai et al., 2000; Shimura et al., 2000; Zhang et al., 2000b). For reasons that are not clear, synphilin-1 shows a higher affinity for parkin that contains familial-associated mutations than the wild-type protein (Chung et al., 2001). Interestingly, these mutations are clustered in the same region of parkin (the IBR and C-terminal R2 ring-finger domains) that interacts preferentially with synphilin-1. The raises the possibility that the lack of Lewy bodies in patients with particular mutations in parkin may be due to sequestration of parkin and synphilin-1 into aggregation- incompetent complexes. Parkin can be detected in some Lewy bodies (Shimura et al., 1999; Choi et al., 2001) and the absence of Lewy bodies in patients with parkin mutations indicates that parkin may be required for Lewy body formation (Mori et al., 1998; Hayashi et al., 2000). These findings potentially link two familial associated PD genes, α-synuclein and parkin, via the synuclein-interacting protein synphilin-1, in the ubiquitination of proteins found in Lewy body-like inclusions. Like synuclein, synphilin-1 is found in Lewy bodies; interestingly, however, synphilin-1 is not found in Lewy neurites (Wakabayashi et al., 2000), suggesting that synphilin-1 is not necessary for the formation of all synuclein-positive aggregates in vivo. In addition, the lack of synphilin-1 in synuclein-positive Lewy neurites that may also contain ubiquitin (Gai et al., 1995; Wakabayashi et al., 1997) raises the still unresolved issue of whether synuclein itself is ubiquitinated, and whether ubiquitin-targeted proteasomal degradation is the mechanism for synuclein turnover (*see* below).

Based on coimmunoprecipitation data and functional analysis, synuclein has been proposed to function as a member of the 14-3-3 family of chaperones, operating as a molecular scaffold that stabilizes the conformations of selected binding partners (Ostrerova et al., 1999). Interestingly, crystal structures for several 14-3-3 isoforms have been deter-

mined. Each 14-3-3 monomer is composed of nine antiparallel alpha helices that form homodimers with a deep large channel in the center of the complex. The walls of this channel contain amphipathic grooves in which peptides or protein domains that contain phosphoserine or phosphothreonine residues can bind (Fu et al., 2000; Obsil et al., 2001). Since coimmunoprecipitation of synuclein with 14-3-3 proteins and their binding partners was performed in the presence of detergent micelles, the results of Ostreova and colleagues raise the possibility that synuclein may structurally resemble this complex in an analogous fashion; that is, by binding to proteins through the formation of extended alpha helices between two synuclein monomers. This could occur under conditions in which synuclein's N-terminal domains form amphipathic alpha helices, such as in the presence of detergent micelles or within cells when synuclein is membrane-bound. Whether synuclein can exist as dimers in membranes and with a similar orientation found in 14-3-3 proteins is unknown, as are questions regarding synuclein's capacity to interact with additional proteins through its amphipathic domains when it is membrane-bound. To determine the specificity of these interactions, it would be interesting to test synuclein's interactions with 14-3-3 family members and their binding partners using purified proteins in the absence and presence of detergent micelles or purified cell membranes.

### *Synuclein Turnover*

The concept that the accumulation of intracellular proteins due to alterations in their folding and/or degradation leads to neurodegeneration in both familial and sporadic cases of PD has received general support. Indeed, genetic and biochemical evidence suggests that ubiquitin-dependent proteasomal degradation may be impaired in a number of neurodegenerative diseases, including PD (see Layfield et al., 2001; McNaught et al., 2001). The presence of polyubiquitin chains rather than ubiquitin monomer in purified Lewy bodies from patients with diffuse Lewy body disease supports this view (Iwatsubo et al., 1996). What remains unclear is whether synuclein itself is polyubiquitinated and if ubiquitin-mediated proteasomal degradation is its normal mode of disposal. Alternatively, if synuclein is not ubiquitinated, is it still degraded by the proteasome, as has been found for some nonubiquitinated proteins (Ciechanover, 1994; Hochstrasser, 1995), or is it degraded in lysosomes or by the other major nonlysosomal proteases, the calpains (Melloni and Pontremoli, 1989; Chan and Mattson, 1999)? Given that synuclein's half-life is almost 2 d (Okochi et al., 2000; Paxinou et al., 2001), acute regulation of synuclein levels is unlikely. Nevertheless, the failure to properly degrade synuclein by whatever mechanism(s) is likely an ultimate cause for its accumulation in numerous Lewy body-containing diseases.

Although a direct interaction between synuclein and synphilin-1 is somewhat clear (Engelender et al., 1999; Chung et al., 2001), synuclein's interaction with parkin is less so. Shimura and colleagues (2001) demonstrated parkin's interaction with a novel 22-kDa O-linked glycosylated form of synuclein from human brain, but not the abundant 14-kDa monomer. In an in vitro ubiquitination assay wild-type parkin, but not AR-JP mutants defective in binding either 22-kDa synuclein or parkin's E2 ubiquitin-conjugating enzyme UbcH7, modified the 22-kDa synuclein into polyubiquitinated high molecular-weight species (Shimura et al., 2001); within cells, these would presumably be targeted for proteasomal degradation. These results suggest that it is the 22-kDa form of synuclein that is a preferred substrate for parkin, and its accumulation in familial AR-PD is the major cause of pathology. Whether 22-kDa synuclein exists in Lewy bodies from idiopathic PD is unknown. In contrast to Shimura and colleagues, only nonglycosylated synuclein could be immunoprecipitated with parkin in human brain extracts and BE-M17 neuroblastoma cells (Choi et al., 2001), and neither form of synuclein associated with parkin in HEK293 cells or the human neuronal cell line SH-SY5Y (Chung et al., 2001), or in rat brain (Fallon et al., 2001). Whether nonglycosylated synuclein in the experiments by Choi and colleagues is also a substrate for parkin's ubiquitin-ligase activity needs to be explored. Recent evidence suggests that parkin contains a PDZ binding motif at its extreme C-terminus, and interacts primarily with Triton X-100 insoluble postsynaptic proteins in rat brain extracts (Fallon et al., 2001). This would seem to indicate that a physiological interaction between parkin and presynaptic synuclein is unlikely. Whether parkin's interaction with synuclein occurs in other parts of the cell is unknown (*see* below). Clearly, this issue needs to be clarified, since the apparent lack of Lewy bodies in AR-JP indicates

that one or more parkin substrates needs to be effectively ubiquinitated/degraded to prevent disease (Mori et al., 1998; Hayashi et al., 2000).

The data from Shimura and colleagues (2001) suggest that synuclein, albeit only its glycosylated form is polyubiquitinated and targeted for degradation by the proteasome. Other investigators, however, have found that synuclein is not ubiquitinated (Ancolio et al., 2000), and synuclein's degradation by the proteasome has been questioned (Bennett et al., 1999; Ancolio et al., 2000; Paxinou et al., 2001; Rideout et al., 2001). In addition, data from a number of studies indicate that in several Lewy body disorders, the number of α-synuclein positive structures exceeds those that stain with anti-ubiquitin antibodies (Spillantini et al., 1998; Gomez-Tortosa et al., 2000; Hurtig et al., 2000). Although comparisons of epitope accessibility between different antibodies are difficult to quantify, especially in cellular aggregates, these results suggest that the accumulation of α-synuclein precedes the appearance of ubiquitin in these structures. Thus, under pathological conditions, synuclein aggregation is likely not the result of a higher propensity for ubiquitinated synuclein to form oligomers/aggregates, but that ubiquitination may be simply a postaggregation cellular response to attempt to eliminate the inclusion. If this turns out to be the case, modification of synuclein by ubiquitin and subsequent degradation by the proteasome may not be its mode of turnover under normal cellular conditions.

Some clues regarding the fate of synuclein may be obtained by determining the location within neurons where synuclein is ultimately degraded. Antibodies to proteasomal subunits label presynaptic boutons in selected nerve populations from rat Central nervous system (CNS) (Mengual et al., 1996), and degradation of cytosolic synaptic proteins can occur locally (Li et al., 1995; Li et al., 1997). This raises the possibility that a proportion of synuclein may be degraded within the synapse. However, optic nerve-ligation experiments suggest that the bulk of synuclein is transported retrogradely on membranes from the synapse to the cell body (Jensen et al., 1999). These membranes may then undergo degradation through the process of autophagy followed by fusion with lysosomes, which is the major mechanism for regulated turnover of cellular organelles (Klionsky and Emr, 2000). Consistent with this, accumulations of synuclein within the cell bodies of primary hippocampal cultures from transgenic mice overexpressing A53T mutant human synuclein colocalized with the lysosomal membrane marker LAMP-1 (Gispert et al., manuscript submitted). Interestingly, no such accumulations could be observed in neurons from mice expressing wild-type human synuclein, suggesting that pathogenic alterations in synuclein's structure may affect its ability to be effectively degraded. Whether these A53T synuclein-containing cell body inclusions are derived from membranes retrogradely transported from the synapse or from reduced anterograde transport needs to be determined. Lysosomes, but not the proteasome have also been implicated in synuclein turnover in HEH293 cells, since treatment with the acidotropic agent ammonium chloride increased the steady-state levels of synuclein, whereas the proteasome inhibitor lactacystin had no effect, nor did lactacystin induce the formation of synuclein-positive ubiquitin-containing aggregates (Paxinou et al., 2001). Whether synuclein accumulates in lysosomes upon treatment with ammonium chloride was not reported.

## Potential Functions of α-synuclein/ Conclusions?

The primary site of α-synuclein function is most likely presynaptic, as it can be isolated from synaptic-membrane fractions and localizes near and around the vesicles of the presynaptic terminal (Clayton and George, 1999). Inactivation of the α-synuclein gene by homologous recombination does not lead to a significant neurological phenotype, although changes in dopaminergic electrophysiology may reflect a specific function related to neurotransmitter release (Abeliovich et al., 2000). When α-synuclein expression is markedly reduced in cultured rat neurons (Murphy et al., 2000), or abolished in α-synuclein knockout mice (Cabin et al., manuscript submitted), the number of vesicles in the distal pool of the presynaptic terminal is reduced. This further suggests a role for synuclein in vesicular dynamics, perhaps through its potential to interact with specific lipids in synaptic membranes. Given synuclein's propensity to bind molecules with high hydrophobic content or exposed hydrophobic domains, a role for synuclein in lipid metabolism appears likely. This could occur either by modulating synaptic lipid content directly or indirectly via the trafficking of membranes either

within the synapse or between the synapse and the cell body.

One difficulty in understanding the function of synuclein is the inherent flexibility of the synuclein monomer and its altered conformation in the presence of lipids. For example, one important question regarding potential synuclein binding partners is whether they bind synuclein in its membrane-bound (modular) or free (natively unfolded) state, or both. Possibly more critical as far as synuclein self-assembly or filament formation is concerned is the whether the stability of these interactions influences the kinetics of synuclein's normal association with membranes, and do alterations in membrane binding play a role in the initiation of aggregates in both familial and idiopathic PD? Investigation into synuclein function may be complicated by the structural alterations that synuclein appears to undergo in the presence of detergent micelles (Weinreb et al., 1996; Eliezer et al., 2001). Since it is unclear whether detergent-associated synuclein structurally mimics its membrane-bound form, coimmunoprecipitation experiments traditionally performed in the presence of these detergents may result in identification of binding partners not normally associated with synuclein physiologically. They may also fail to reveal bona-fide synuclein binding partners for similar reasons. Likewise, although the unstructured acidic C-terminus of synuclein is a likely site for interaction with additional proteins, nonspecific electrostatic interactions might lead to potentially false avenues of investigation. Therefore, persuasive evidence of a role for synuclein in any particular pathway or function likely requires multiple approaches.

Transgenic animal models of PD based on synuclein overexpression have so far revealed little in the way of understanding synuclein's normal function. However, suppressor analysis from transgenic flies expressing wild-type or PD mutant synucleins (*see* Feany and Bender, 2000) will likely provide valuable information on mechanisms of synuclein aggregation and toxicity, as well as significant insight as to why wild-type synuclein in vertebrates remains for the most part soluble under nonpathological conditions. Similarly, synuclein expression in lower eukaryotes (e.g., yeast) may provide clues as to its normal function, as well as how its dysregulation may affect neuronal physiology in higher organisms.

The discovery of synuclein mutations in familial PD marked a turning point of cultural change in the study of PD pathogenesis. As such, it is an example what is becoming more common in neurodegenerative disease research: giant leaps forward in genetic discovery that demand careful and more time-consuming follow-up of the cell biology of proteins implicated by such discovery. While therapeutic strategies for PD initially revolve around understanding synuclein aggregation, a clearer understanding of the basic cell biology of this protein will advance our understanding of the cellular pathways involved in PD and the role of other proteins that have since been implicated through genetic findings, and define a system for which the impact of environmental triggers for PD may be more systematically evaluated.

## References

Abeliovich A., Schmitz Y., Farinas I., Choi-Lundberg D., Ho W. H., Castillo P. E., et al. (2000) Mice lacking alpha-synuclein display functional deficits in the nigrostriatal dopamine system. *Neuron* **25,** 239–252.

Ancolio K., Alves da Costa C., Ueda K., and Checler F. (2000) Alpha-synuclein and the Parkinson's disease-related mutant Ala53Thr- alpha-synuclein do not undergo proteasomal degradation in HEK293 and neuronal cells. *Neurosci. Lett.* **285,** 79–82.

Arima K., Ueda K., Sunohara N., Hirai S., Izumiyama Y., Tonozuka-Uehara H., and Kawai M. (1998) Immunoelectron-microscopic demonstration of NACP/alpha-synuclein-epitopes on the filamentous component of Lewy bodies in Parkinson's disease and in dementia with Lewy bodies. *Brain Res.* **808,** 93–100.

Baba M., Nakajo S., Tu P. H., Tomita T., Nakaya K., Lee V. M., et al. (1998) Aggregation of alpha-synuclein in Lewy bodies of sporadic Parkinson's disease and dementia with Lewy bodies. *Am. J. Pathol.* **152,** 879–884.

Barzilai A., Melamed E., and Shirvan A. (2001) Is there a rationale for neuroprotection against dopamine toxicity in Parkinson's disease? *Cell Mol. Neurobiol.* **21,** 215–235.

Bayer T. A., Jakala P., Hartmann T., Egensperger R., Buslei R., Falkai P., and Beyreuther K. (1999) Neural expression profile of alpha-synuclein in developing human cortex. *Neuroreport* **10,** 2799–2803.

Bennett M. C., Bishop J. F., Leng Y., Chock P. B., Chase T. N., and Mouradian M. M. (1999) Degradation of alpha-synuclein by proteasome. *J. Biol. Chem.* **274,** 33,855–33,858.

Betarbet R., Sherer T. B., MacKenzie G., Garcia-Osuna M., Panov A. V., and Greenamyre J. T. (2000) Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nat. Neurosci.* **3,** 1301–1306.

Biere A. L., Wood S. J., Wypych J., Steavenson S., Jiang Y., Anafi D., et al. (2000) Parkinson's disease-associated alpha-synuclein is more fibrillogenic than beta- and gamma-synuclein and cannot cross-seed its homologs. *J. Biol. Chem.* **275,** 34,574–34,579.

Campbell B. C., McLean C. A., Culvenor J. G., Gai W. P., Blumbergs P. C., Jakala P., et al. (2001) The solubility of alpha-synuclein in multiple system atrophy differs from that of dementia with Lewy bodies and Parkinson's disease. *J. Neurochem.* **76,** 87–96.

Chan S. L. and Mattson M. P. (1999) Caspase and calpain substrates: roles in synaptic plasticity and cell death. *J. Neurosci. Res.* **58,** 167–190.

Choi P., Golts N., Snyder H., Chong M., Petrucelli L., Hardy J., et al. (2001) Co-association of parkin and alpha-synuclein. *Neuroreport* **12,** 2839–2843.

Choinowski T., Hauser H., and Piontek K. (2000) Structure of sterol carrier protein 2 at 1.8 A resolution reveals a hydrophobic tunnel suitable for lipid binding. *Biochemistry* **39,** 1897–1902.

Chung K. K., Zhang Y., Lim K. L., Tanaka Y., Huang H., Gao J., et al. (2001) Parkin ubiquitinates the alpha-synuclein-interacting protein, synphilin-1: implications for Lewy-body formation in Parkinson disease. *Nat. Med.* **7,** 1144–1150.

Ciechanover A. (1994) The ubiquitin-proteasome proteolytic pathway. *Cell* **79,** 13–21.

Clayton D. F. and George J. M. (1998) The synucleins: a family of proteins involved in synaptic function, plasticity, neurodegeneration and disease. *Trends Neurosci.* **21,** 249–254.

Clayton D. F. and George J. M. (1999) Synucleins in synaptic plasticity and neurodegenerative disorders. *J. Neurosci. Res.* **58,** 120–129.

Cole N. B., Murphy D. D., Grider T., et al. (2002) Lipid droplet binding and oligomeric properties of the Parkinson's disease protein alpha-synuclein. *J. Biol. Chem.* **277,** 6344–6352.

Conway K. A., Lee S. J., Rochet J. C., Ding T. T., Williamson R. E., and Lansbury P. T., Jr. (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. *Proc. Natl. Acad. Sci. USA* **97,** 571–576.

Conway K. A., Rochet J. C., Bieganski R. M., and Lansbury Jr P. T. (2001) Kinetic stabilization of the alpha synuclein protofibril by a dopamine-alpha-synuclein adduct. *Science* **294,** 1346–1349.

Crowther R. A., Jakes R., Spillantini M. G., and Goedert M. (1998) Synthetic filaments assembled from C-terminally truncated alpha- synuclein. *FEBS Lett.* **436,** 309–312.

Davidson W. S., Jonas A., Clayton D. F., and George J. M. (1998) Stabilization of alpha-synuclein secondary structure upon binding to synthetic membranes. *J. Biol. Chem.* **273,** 9443–9449.

Deutsch J. W. and Kelly R. B. (1981) Lipids of synaptic vesicles: relevance to the mechanism of membrane fusion. *Biochemistry* **20,** 378–385.

Eliezer D., Kutluay E., Bussell R. Jr., and Browne, G. (2001) Conformational properties of alpha-synuclein in its free and lipid- associated states. *J. Mol. Biol.* **307,** 1061–1073.

Ellis C. E., Schwartzberg P. L., Grider T. L., Fink D. W., and Nussbaum R. L. (2000) Alpha-synuclein is phosphorylated by members of the Src family of protein tyrosine kinases. *J. Biol. Chem.* **14,** 14.

Engelender S., Kaminsky Z., Guo X., Sharp A. H., Amaravi R. K., Kleiderlein J. J., et al. (1999) Synphilin-1 associates with alpha-synuclein and promotes the formation of cytosolic inclusions. *Nat. Genet.* **22,** 110–114.

Fallon L., Moreau F., Croft B. G., Labib N., Gu W. J., and Fon E. A. (2001) Parkin and CASK/LIN-2 associate via a PDZ-mediated interaction and are co-localized in lipid rafts and postsynaptic densities in brain. *J. Biol. Chem.* **25,** 25.

Farrer M., Maraganore D. M., Lockhart P., Singleton A., Lesnick T. G., de Andrade M., et al. (2001) alpha-Synuclein gene haplotypes are associated with Parkinson's disease. *Hum. Mol. Genet.* **10,** 1847–1851.

Feany M. B. and Bender W. W. (2000) A Drosophila model of Parkinson's disease. *Nature* **404,** 394–398.

Fu H., Subramanian R. R., and Masters S. C. (2000) 14-3-3 proteins: structure, function, and regulation. *Annu. Rev. Pharmacol. Toxicol.* **40,** 617–647.

Gai W. P., Blessing W. W., and Blumbergs P. C. (1995) Ubiquitin-positive degenerating neurites in the brainstem in Parkinson's disease. *Brain* **118,** 1447–1459.

Gai W. P., Yuan H. X., Li X. Q., Power J. T., Blumbergs P. C., and Jensen P. H. (2000) In situ and in vitro study of colocalization and segregation of alpha-synuclein, ubiquitin, and lipids in Lewy bodies. *Exp. Neurol.* **166,** 324–333.

Galvin J. E., Schuck T. M., Lee V. M., and Trojanowski J. Q. (2001) Differential expression and distribution of alpha-, beta-, and gamma-synuclein in the developing human substantia nigra. *Exp. Neurol.* **168,** 347–355.

George J. M., Jin H., Woods W. S., and Clayton D. F. (1995) Characterization of a novel protein regulated during the critical period for song learning in the zebra finch. *Neuron* **15,** 361–372.

Giasson B. I., Murray I. V., Trojanowski J. Q., and Lee V. M. (2001) A hydrophobic stretch of 12 amino acid residues in the middle of alpha-synuclein is essential for filament assembly. *J. Biol. Chem.* **276,** 2380–2386.

Goedert M. (2001) Alpha-synuclein and neurodegenerative diseases. *Nat. Rev. Neurosci.* **2,** 492–501.

Goldberg M. S. and Lansbury P. T. Jr. (2000) Is there a cause-and-effect relationship between alpha-synuclein fibrillization and Parkinson's disease? *Nat. Cell Biol.* **2,** E115–119.

Gomez-Tortosa E., Newell K., Irizarry M. C., Sanders J. L., and Hyman B. T. (2000) alpha-Synuclein immunoreactivity in dementia with Lewy bodies: morphological staging and comparison with ubiquitin immunostaining. *Acta Neuropathol. (Berl)* **99,** 352–357.

Hardy J. and Gwinn-Hardy K. (1998) Genetic classification of primary neurodegenerative disease. *Science* **282,** 1075–1079.

Hashimoto M., Hsu L. J., Sisk A., Xia Y., Takeda A., Sundsmo M., and Masliah E. (1998) Human recombinant NACP/alpha-synuclein is aggregated and fibrillated in vitro: relevance for Lewy body disease. *Brain Res.* **799,** 301–306.

Hashimoto M., Yoshimoto M., Sisk A., Hsu L. J., Sundsmo M., Kittel A., et al. (1997) NACP, a synaptic protein involved in Alzheimer's disease, is differentially regulated during megakaryocyte differentiation. *Biochem. Biophys. Res. Commun.* **237,** 611–616.

Hayashi S., Wakabayashi K., Ishikawa A., Nagai H., Saito M., Maruyama M., et al. (2000) An autopsy case of autosomal-recessive juvenile parkinsonism with a homozygous exon 4 deletion in the parkin gene. *Mov. Disord.* **15,** 884–888.

Hertzel A. V. and Bernlohr D. A. (2000) The mammalian fatty acid-binding protein multigene family: molecular and genetic insights into function. *Trends Endocrinol. Metab.* **11,** 175–180.

Hochstrasser M. (1995) Ubiquitin, proteasomes, and the regulation of intracellular protein degradation. *Curr. Opin. Cell Biol.* **7,** 215–223.

Hsu L. J., Mallory M., Xia Y., Veinbergs I., Hashimoto M., Yoshimoto M., et al. (1998) Expression pattern of synucleins (non-Abeta component of Alzheimer's disease amyloid precursor protein/alpha-synuclein) during murine brain development. *J. Neurochem.* **71,** 338–344.

Hurtig H. I., Trojanowski J. Q., Galvin J., Ewbank D., Schmidt M. L., Lee V. M., et al. (2000) Alpha-synuclein cortical Lewy bodies correlate with dementia in Parkinson's disease. *Neurology* **54,** 1916–1921.

Imai Y., Soda M., and Takahashi R. (2000) Parkin suppresses unfolded protein stress-induced cell death through its E3 ubiquitin-protein ligase activity. *J. Biol. Chem.* **275,** 35,661–35,664.

Irizarry M. C., Kim T. W., McNamara M., Tanzi R. E., George J. M., Clayton D. F., and Hyman B. T. (1996) Characterization of the precursor protein of the non-A beta component of senile plaques (NACP) in the human central nervous system. *J. Neuropathol. Exp. Neurol.* **55,** 889–895.

Iwai A., Masliah E., Yoshimoto M., Ge N., Flanagan L., de Silva H. A., et al. (1995) The precursor protein of non-Abeta component of Alzheimer's disease amyloid is a presynaptic protein of the central nervous system. *Neuron* **14,** 467–475.

Iwatsubo T., Yamaguchi H., Fujimuro M., Yokosawa H., Ihara Y., Trojanowski J. Q., and Lee V. M. (1996) Purification and characterization of Lewy bodies from the brains of patients with diffuse Lewy body disease. *Am. J. Pathol.* **148,** 1517–1529.

Jakes R., Spillantini M. G., and Goedert M. (1994) Identification of two distinct synucleins from human brain. *FEBS Lett.* **345,** 27–32.

Jenco J. M., Rawlingson A., Daniels B., and Morris A. J. (1998) Regulation of phospholipase D2: selective inhibition of mammalian phospholipase D isoenzymes by alpha- and beta-synucleins. *Biochemistry* **37,** 4901–4909.

Jensen P. H., Hojrup P., Hager H., Nielsen M. S., Jacobsen L., Olesen O. F., et al. (1997) Binding of Abeta to alpha- and beta-synucleins: identification of segments in alpha-synuclein/NAC precursor that bind Abeta and NAC. *Biochem. J.* **323,** 539–546.

Jensen P. H., Li J. Y., Dahlstrom A., and Dotti C. G. (1999) Axonal transport of synucleins is mediated by all rate components. *Eur. J. Neurosci.* **11,** 3369–3376.

Jo E., McLaurin J., Yip C. M., St George-Hyslop P., and Fraser P. E. (2000) alpha-Synuclein membrane interactions and lipid specificity. *J. Biol. Chem.* **275,** 34,328–34,334.

Kahle P. J., Neumann M., Ozmen L., Muller V., Jacobsen H., Schindzielorz A., et al. (2000) Subcellular localization of wild-type and Parkinson's disease-associated mutant alpha-synuclein in human and transgenic mouse brain. *J. Neurosci.* **20,** 6365–6373.

Kim T. D., Paik S. R., Yang C. H., and Kim J. (2000) Structural changes in alpha-synuclein affect its

chaperone-like activity in vitro. *Protein Sci.* **9,** 2489–2496.

Kitada T., Asakawa S., Hattori N., Matsumine H., Yamamura Y., Minoshima S., et al. (1998) Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature* **392,** 605–608.

Klionsky D. J. and Emr S. D. (2000) Autophagy as a regulated pathway of cellular degradation. *Science* **290,** 1717–1721.

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. *Nat. Genet.* **18,** 106–108.

Kruger R., Vieira-Saecker A. M., Kuhn W., Berg D., Muller T., Kuhnl N., et al. (1999) Increased susceptibility to sporadic Parkinson's disease by a certain combined alpha-synuclein/apolipoprotein E genotype. *Ann. Neurol.* **45,** 611–617.

Langston J. W., Sastry S., Chan P., Forno L. S., Bolin L. M., and Di Monte D. A. (1998) Novel alpha-synuclein-immunoreactive proteins in brain samples from the Contursi kindred, Parkinson's, and Alzheimer's disease. *Exp. Neurol.* **154,** 684–690.

Lavedan C. (1998) The synuclein family. *Genome Res.* **8,** 871–880.

Lavedan C., Leroy E., Dehejia A., Buchholtz S., Dutra A., Nussbaum R. L., and Polymeropoulos M. H. (1998) Identification, localization and characterization of the human gamma-synuclein gene. *Hum. Genet.* **103,** 106–112.

Layfield R., Alban A., Mayer R. J., and Lowe J. (2001) The ubiquitin protein catabolic disorders. *Neuropathol. Appl. Neurobiol.* **27,** 171–179.

Lee D., Lee E. K., Lee J. H., Chang C. S., and Paik S. R. (2001a) Self-oligomerization and protein aggregation of alpha-synuclein in the presence of Coomassie Brilliant Blue. *Eur. J. Biochem.* **268,** 295–301.

Lee F. J., Liu F., Pristupa Z. B., and Niznik H. B. (2001b) Direct binding and functional coupling of alpha-synuclein to the dopamine transporters accelerate dopamine-induced apoptosis. *FASEB J.* **15,** 916–926.

Lee H. J., Choi C., and Lee S. J. (2001c) Membrane-bound alpha-synuclein has a high aggregation propensity and the ability to seed the aggregation of cytosolic form. *J. Biol. Chem.* **25,** 25.

Li J. Y., De Camilli P., and Dahlstrom A. (1997) Intraneuronal trafficking and distribution of amphiphysin and synaptojanin in the rat peripheral nervous system and the spinal cord. *Eur. J. Neurosci.* **9,** 1864–1874.

Li J. Y., Jahn R., and Dahlstrom A. (1995) Rab3a, a small GTP-binding protein, undergoes fast anterograde transport but not retrograde transport in neurons. *Eur. J. Cell Biol.* **67,** 297–307.

Maroteaux L., Campanelli J. T., and Scheller R. H. (1988) Synuclein: a neuron-specific protein localized to the nucleus and presynaptic nerve terminal. *J. Neurosci.* **8,** 2804–2815.

Mattson M. P. (1998) Modification of ion homeostasis by lipid peroxidation: roles in neuronal degeneration and adaptive plasticity. *Trends Neurosci.* **21,** 53–57.

McNaught K. S., Olanow C. W., Halliwell B., Isacson O., and Jenner P. (2001) Failure of the ubiquitin-proteasome system in Parkinson's disease. *Nat. Rev. Neurosci.* **2,** 589–594.

Melloni E. and Pontremoli S. (1989) The calpains. *Trends Neurosci.* **12,** 438–444.

Mengual E., Arizti P., Rodrigo J., Gimenez-Amaya J. M., and Castano J. G. (1996) Immunohistochemical distribution and electron microscopic subcellular localization of the proteasome in the rat CNS. *J. Neurosci.* **16,** 6331–6341.

Mishra V. K. and Palgunachari M. N. (1996) Interaction of model class A1, class A2, and class Y amphipathic helical peptides with membranes. *Biochemistry* **35,** 11210–11220.

Moore K. and Roberts L. J. 2nd (1998) Measurement of lipid peroxidation. *Free Radic. Res.* **28,** 659–671.

Mori H., Kondo T., Yokochi M., Matsumine H., Nakagawa-Hattori Y., Miyake T., et al. (1998) Pathologic and biochemical studies of juvenile parkinsonism linked to chromosome 6q. *Neurology* **51,** 890–892.

Murphy D. D., Rueter S. M., Trojanowski J. Q., and Lee V. M. (2000) Synucleins are developmentally expressed, and alpha-synuclein regulates the size of the presynaptic vesicular pool in primary hippocampal neurons. *J. Neurosci.* **20,** 3214–3220.

Nakamura T., Yamashita H., Takahashi T., and Nakamura S. (2001) Activated Fyn phosphorylates alpha-synuclein at tyrosine residue 125. *Biochem. Biophys. Res. Commun.* **280,** 1085–1092.

Narayanan V. and Scarlata S. (2001) Membrane binding and self-association of alpha-synucleins. *Biochemistry* **40,** 9927–9934.

Neystat M., Lynch T., Przedborski S., Kholodilov N., Rzhetskaya M., and Burke R. E. (1999) Alpha-synuclein expression in substantia nigra and cortex in Parkinson's disease. *Mov. Disord.* **14,** 417–422.

Obsil T., Ghirlando R., Klein D. C., Ganguly S., and Dyda F. (2001) Crystal structure of the 14-3-3zeta:serotonin N-acetyltransferase complex. a role for scaffolding in enzyme regulation. *Cell* **105,** 257–267.

Okochi M., Walter J., Koyama A., Nakajo S., Baba M., Iwatsubo T., Meijer L., Kahle P. J., and Haass C. (2000) Constitutive phosphorylation of the Parkin-

son's disease associated alpha-synuclein. *J. Biol. Chem.* **275,** 390–397.

Osborne S. L., Meunier F. A., and Schiavo G. (2001) Phosphoinositides as key regulators of synaptic function. *Neuron* **32,** 9–12.

Ostrerova N., Petrucelli L., Farrer M., Mehta N., Choi P., Hardy J., and Wolozin B. (1999) alpha-Synuclein shares physical and functional homology with 14-3-3 proteins. *J. Neurosci.* **19,** 5782–5791.

Paik S. R., Lee J. H., Kim D. H., Chang C. S., and Kim Y. S. (1998) Self-oligomerization of NACP, the precursor protein of the non-amyloid beta/A4 protein (A beta) component of Alzheimer's disease amyloid, observed in the presence of a C-terminal A beta fragment (residues 25- 35). *FEBS Lett.* **421,** 73–76.

Paik S. R., Shin H. J., Lee J. H., Chang C. S., and Kim J. (1999) Copper(II)-induced self-oligomerization of alpha-synuclein. *Biochem. J.* **340,** 821–828.

Paxinou E., Chen Q., Weisse M., Giasson B. I., Norris E. H., Rueter S. M., et al. (2001) Induction of alpha-synuclein aggregation by intracellular nitrative insult. *J. Neurosci.* **21,** 8053–8061.

Perrin R. J., Woods W. S., Clayton D. F., and George J. M. (2000) Interaction of human alpha-Synuclein and Parkinson's disease variants with phospholipids. Structural analysis using site-directed mutagenesis. *J. Biol. Chem.* **275,** 34,393–34,398.

Perrin R. J., Woods W. S., Clayton D. F., and George J. M. (2001) Exposure to long chain polyunsaturated fatty acids triggers rapid multimerization of synucleins. *J. Biol. Chem.* **276,** 41,958–41,962.

Petersen K., Olesen O. F., and Mikkelsen J. D. (1999) Developmental expression of alpha-synuclein in rat hippocampus and cerebral cortex. *Neuroscience* **91,** 651–659.

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.

Pronin A. N., Morris A. J., Surguchov A., and Benovic J. L. (2000) Synucleins are a novel class of substrates for G protein-coupled receptor kinases. *J. Biol. Chem.* **275,** 26,515–26,522.

Refsgaard H. H., Tsai L., and Stadtman E. R. (2000) Modifications of proteins by polyunsaturated fatty acid peroxidation products. *Proc. Natl. Acad. Sci. USA* **97,** 611–616.

Rideout H. J., Larsen K. E., Sulzer D., and Stefanis L. (2001) Proteasomal inhibition leads to formation of ubiquitin/alpha-synuclein-immunoreactive inclusions in PC12 cells. *J. Neurochem.* **78,** 899–908.

Rockenstein E., Hansen L. A., Mallory M., Trojanowski J. Q., Galasko D., and Masliah E. (2001) Altered expression of the synuclein family mRNA in Lewy body and Alzheimer's disease. *Brain Res.* **914,** 48–56.

Sharon R., Goldberg M. S., Bar-Josef I., Betensky R. A., Shen J., and Selkoe D. J. (2001) alpha-Synuclein occurs in lipid-rich high molecular weight complexes, binds fatty acids, and shows homology to the fatty acid-binding proteins. *Proc. Natl. Acad. Sci. USA* **98,** 9110–9115.

Shibayama-Imazu T., Okahashi I., Omata K., Nakajo S., Ochiai H., Nakai Y., et al. (1993) Cell and tissue distribution and developmental change of neuron specific 14 kDa protein (phosphoneuroprotein 14). *Brain Res.* **622,** 17–25.

Shimura H., Hattori N., Kubo S., Mizuno Y., Asakawa S., Minoshima S., et al. (2000) Familial Parkinson disease gene product, parkin, is a ubiquitin-protein ligase. *Nat. Genet.* **25,** 302–305.

Shimura H., Hattori N., Kubo S., Yoshikawa M., Kitada T., Matsumine H., et al. (1999) Immunohistochemical and subcellular localization of Parkin protein: absence of protein in autosomal recessive juvenile parkinsonism patients. *Ann. Neurol.* **45,** 668–672.

Shimura H., Schlossmacher M. G., Hattori N., Frosch M. P., Trockenbacher A., Schneider R., et al. (2001) Ubiquitination of a new form of alpha-synuclein by parkin from human brain: implications for Parkinson's disease. *Science* **293,** 263–269.

Shin H. J., Lee E. K., Lee J. H., Lee D., Chang C. S., Kim Y. S., and Paik S. R. (2000) Eosin interaction of alpha-synuclein leading to protein self-oligomerization. *Biochim. Biophys. Acta* **1481,** 139–146.

Souza J. M., Giasson B. I., Chen Q., Lee V. M., and Ischiropoulos H. (2000a) Dityrosine cross-linking promotes formation of stable alpha-synuclein polymers. Implication of nitrative and oxidative stress in the pathogenesis of neurodegenerative synucleinopathies. *J. Biol. Chem.* **275,** 18,344–18,349.

Souza J. M., Giasson B. I., Lee V. M., and Ischiropoulos H. (2000b) Chaperone-like activity of synucleins. *FEBS Lett.* **474,** 116–119.

Spillantini M. G., Crowther R. A., Jakes R., Hasegawa M., and Goedert M. (1998) alpha-Synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with lewy bodies. *Proc. Natl. Acad. Sci. USA* **95,** 6469–6473.

Spillantini M. G., Schmidt M. L., Lee V. M., Trojanowski J. Q., Jakes R., and Goedert M. (1997) Alpha-synuclein in Lewy bodies. *Nature* **388,** 839–840.

Stefanis L., Kholodilov N., Rideout H. J., Burke R. E., and Greene L. A. (2001) Synuclein-1 is selectively up-regulated in response to nerve growth factor treatment in PC12 cells. *J. Neurochem.* **76,** 1165–1176.

Storch J. and Thumser A. E. (2000) The fatty acid transport function of fatty acid-binding proteins. *Biochim. Biophys. Acta* **1486,** 28–44.

Surguchov A., Surgucheva I., Solessio E., and Baehr W. (1999) Synoretin: a new protein belonging to the synuclein family. *Mol. Cell Neurosci.* **13,** 95–103.

Tan E. K., Matsuura T., Nagamitsu S., Khajavi M., Jankovic J., and Ashizawa T. (2000) Polymorphism of NACP-Rep1 in Parkinson's disease: an etiologic link with essential tremor? *Neurology* **54,** 1195–1198.

Tanji K., Imaizumi T., Yoshida H., Mori F., Yoshimoto M., Satoh K., and Wakabayashi K. (2001) Expression of alpha-synuclein in a human glioma cell line and its up- regulation by interleukin-1beta. *Neuroreport* **12,** 1909–1912.

Touchman J. W., Dehejia A., Chiba-Falek O., Cabin D. E., Schwartz J. R., Orrison B. M., et al. (2001) Human and mouse alpha-synuclein genes: comparative genomic sequence analysis and identification of a novel gene regulatory element. *Genome Res.* **11,** 78–86.

Ueda K., Fukushima H., Masliah E., Xia Y., Iwai A., Yoshimoto M., et al. (1993) Molecular cloning of cDNA encoding an unrecognized component of amyloid in Alzheimer disease. *Proc. Natl. Acad. Sci. USA* **90,** 11,282–11,286.

Uversky V. N., Gillespie J. R., and Fink A. L. (2000) Why are "natively unfolded" proteins unstructured under physiologic conditions? *Proteins* **41,** 415–427.

Uversky V. N., Li J., and Fink A. L. (2001a) Evidence for a partially folded intermediate in alpha-synuclein fibril formation. *J. Biol. Chem.* **276,** 10737–10744.

Uversky V. N., Li J., and Fink A. L. (2001b) Metal-triggered structural transformations, aggregation and fibrillation of human alpha-synuclein. A possible molecular link between parkinson's disease and heavy metal exposure. *J. Biol. Chem.* **276,** 44284–44296.

Uversky V. N., Li J., and Fink A. L. (2001c) Pesticides directly accelerate the rate of alpha-synuclein fibril formation: a possible factor in Parkinson's disease. *FEBS Lett.* **500,** 105–108.

Vila M., Vukosavic S., Jackson-Lewis V., Neystat M., Jakowec M., and Przedborski S. (2000) Alpha-synuclein up-regulation in substantia nigra dopaminergic neurons following administration of the parkinsonian toxin MPTP. *J. Neurochem.* **74,** 721–729.

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

Wakabayashi K., Engelender S., Yoshimoto M., Tsuji S., Ross C. A., and Takahashi H. (2000) Synphilin-1 is present in Lewy bodies in Parkinson's disease. *Ann. Neurol.* **47,** 521–523.

Wakabayashi K., Matsumoto K., Takayama K., Yoshimoto M., and Takahashi H. (1997) NACP, a presynaptic protein, immunoreactivity in Lewy bodies in Parkinson's disease. *Neurosci. Lett.* **239,** 45–48.

Weinreb P. H., Zhen W., Poon A. W., Conway K. A., and Lansbury P. T. Jr. (1996) NACP, a protein implicated in Alzheimer's disease and learning, is natively unfolded. *Biochemistry* **35,** 13709–13715.

Withers G. S., George J. M., Banker G. A., and Clayton D. F. (1997) Delayed localization of synelfin (synuclein, NACP) to presynaptic terminals in cultured rat hippocampal neurons. *Brain Res. Dev. Brain Res.* **99,** 87–94.

Wood S. J., Wypych J., Steavenson S., Louis J. C., Citron M., and Biere A. L. (1999) alpha-synuclein fibrillogenesis is nucleation-dependent. Implications for the pathogenesis of Parkinson's disease. *J. Biol. Chem.* **274,** 19,509–19,512.

Xia Y., Rohan de Silva H. A., Rosi B. L., Yamaoka L. H., Rimmler J. B., Pericak-Vance M. A., et al. (1996) Genetic studies in Alzheimer's disease with an NACP/alpha-synuclein polymorphism. *Ann. Neurol.* **40,** 207–215.

Zhang Y., Dawson V. L., and Dawson T. M. (2000a) Oxidative stress and genetics in the pathogenesis of Parkinson's disease. *Neurobiol. Dis.* **7,** 240–250.

Zhang Y., Gao J., Chung K. K., Huang H., Dawson V. L., and Dawson T. M. (2000b) Parkin functions as an E2-dependent ubiquitin- protein ligase and promotes the degradation of the synaptic vesicle-associated protein, CDCrel-1. *Proc. Natl. Acad. Sci. USA* **97,** 13354–13359.