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α-Synuclein: Membrane Interactions and Toxicity in Parkinson's Disease

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protein folding, vesicle trafficking, neurodegeneration

Abstract

In the late 1990s, mutations in the synaptic protein α-synuclein (α-syn) were identified in families with hereditary Parkinson's disease (PD). Rapidly, α-syn became the target of numerous investigations that have transformed our understanding of the pathogenesis underlying this disorder. α-Syn is the major component of Lewy bodies (LBs), cytoplasmic protein aggregates that form in the neurons of PD patients. α-Syn interacts with lipid membranes and adopts amyloid conformations that deposit within LBs. Work in yeast and other model systems has revealed that α-syn-associated toxicity might be the consequence of abnormal membrane interactions and alterations in vesicle trafficking. Here we review evidence regarding α-syn's normal interactions with membranes and regulation of synaptic vesicles as well as how overexpression of α-syn yields global cellular dysfunction. Finally, we present a model linking vesicle dynamics to toxicity with the sincere hope that understanding these disease mechanisms will lead to the development of novel, potent therapeutics.

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INTRODUCTION

The ability of proteins to fold properly and adopt appropriate tertiary structures is critical to their proper localization and function. Abnormalities in protein folding can impair protein function in many different ways: (*a*) Improperly folded proteins may simply be degraded before they reach their final destination; (*b*) misfolded proteins may be unable to execute their tasks efficiently either through defects within the protein itself or indirectly through altered interactions with other proteins; and (*c*) misfolding can confer new and toxic gain-of-function effects. Therefore, it is not surprising that many catastrophic diseases are associated with protein misfolding. Parkinson's disease (PD) is one such disorder, afflicting approximately 6 million individuals worldwide (**http://michaeljfox.org/**).

The protein α-synuclein (α-syn) is of particular interest because of its close association with PD and related neurodegenerative disorders. α-Syn is misfolded and deposited within insoluble protein aggregates in both sporadic and dominant familial forms of the disease. Thus, α-syn dysfunction appears to be a critical determinant for the development of PD. Over the past dozen years, many strides have been made in understanding α-syn's role in health and disease. Although the function of α-syn is still unclear, substantial evidence now exists suggesting that α-syn interacts directly with phospholipid membranes, particularly those of vesicles. In fact, α-syn appears to be a critical regulator of vesicle dynamics at the synapse.

This review begins with a description of the diseases in which α-syn misfolding and/or dysfunction is involved. We then examine α-syn's biophysical properties and how they favor lipid interactions both in vitro and in vivo. Finally, we review emerging data from yeast and other model organisms demonstrating that α-syn toxicity is associated with deficits in vesicle trafficking, mitochondrial function, and lipid/sterol biosynthesis. A better understanding of this cellular dysfunction will enable the development of novel therapeutic strategies

capable of addressing the diverse biological defects underlying PD pathogenesis.

AN OVERVIEW OF PARKINSON'S DISEASE, PARKINSONISM, AND α-SYNUCLEINOPATHIES

PD is clinically characterized by bradykinesia, resting tremor, and postural rigidity. As the disease progresses, patients frequently develop cognitive impairment and depression. Most motor symptoms can be attributed to the degeneration of dopaminergic neurons within the substantia nigra pars compacta, a key regulatory nucleus of basal ganglia circuitry. However, it is now increasingly appreciated that several other nondopaminergic neuronal populations also degenerate. These include various autonomic nuclei and the locus ceruleus as well as glutamatergic neurons throughout the cerebral cortex.

Several other neurodegenerative diseases—collectively known as parkinsonian disorders—share the clinical presentation of PD (i.e., parkinsonism). However, these diseases are pathologically distinct entities with different histopathological hallmarks and patterns of neuronal degeneration. PD is distinguished from other forms of parkinsonism by the presence of Lewy bodies (LBs) and Lewy neurites (LNs), which are juxtanuclear and neuritic ubiquitinated protein aggregates composed predominantly of the synaptic protein α-syn (**Figure 1*a***). In contrast, parkinsonian

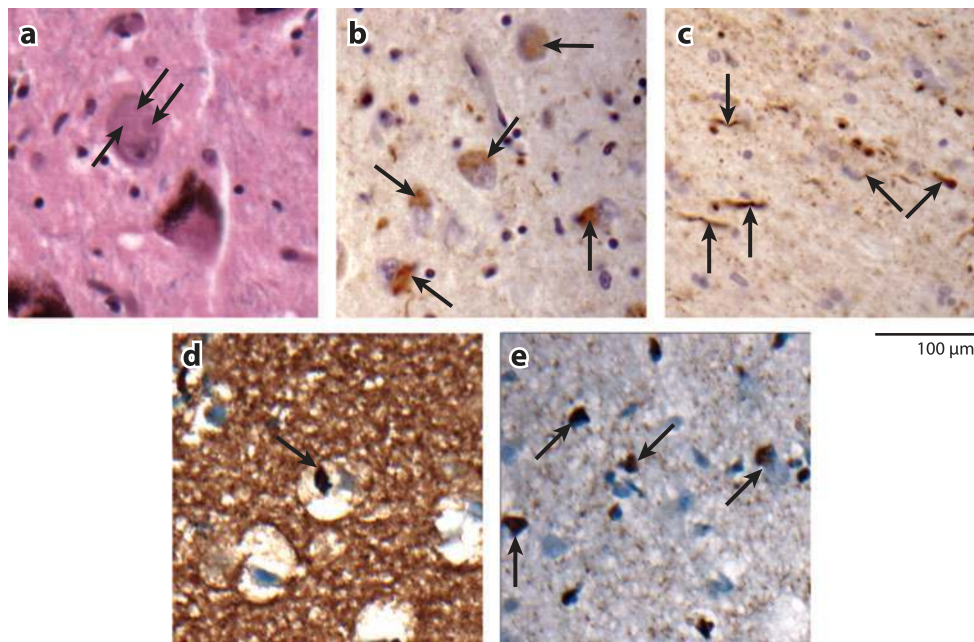

Figure 1

α-Synuclein (α-syn) is the major component of Lewy bodies (LBs) and Lewy neurites (LNs) in Parkinson's disease (PD) and dementia with LBs (DLB) and of neuronal and glial cytoplasmic inclusions in multiple system atrophy (MSA). (*a*) A luxol fast blue-, hematoxylin-, and eosin-stained section of the substantia nigra from a patient with PD. Three LBs (*arrows*) are present in the cytoplasm of a swollen dopaminergic neuron. Nearby is a normally pigmented dopaminergic neuron. (*b*,*c*) Immunohistochemistry for α-syn on a section of the amygdala from a patient with DLB. Numerous cortical LBs (*b*, *arrows*) and LNs (*c*, *arrows*) are labeled by the anti-α-syn antibody. (*d*,*e*) Immunohistochemistry for α-syn on a section of the putamen from a patient with MSA. A single neuronal cytoplasmic inclusion (*d*, *arrow*) is visible in a relatively preserved area of the putamen. The preserved neuropil is also reactive for α-syn. Several glial cytoplasmic inclusions (*e*, *arrows*) are present in severely degenerated areas of the putamen.

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disorders such as progressive supranuclear palsy, corticobasal degeneration, and dementia pugilistica feature neurofibrillary tangle pathology containing the microtubule-binding protein Tau, not α-syn.

α-Syn, in addition to accumulating in LBs and LNs in PD, also accumulates in some dementing disorders, namely dementia with LBs (DLB) and multiple system atrophy (MSA) (**Figure 1*b–e***). Clinically and pathologically, DLB overlaps significantly with PD. Patients often have some parkinsonian symptoms, but they also have a rapidly progressing dementia. These patients often require significant assistance in performing even the most basic activities of daily living. Pathologically, DLB is characterized by mild, diffuse neuronal loss and the presence of cortical LBs (**Figure 1*b***) and LNs (**Figure 1*c***) throughout the amygdala, entorhinal cortex, and neocortex in addition to the brain stem pathology that typifies PD. PD and DLB may be considered to be spectrums of the same disorder.

MSA is an aggressive neurodegenerative disorder that is pathologically distinct from PD and DLB. Patients present with parkinsonism, dementia, and/or ataxia. At autopsy there is devastating degeneration of the striatum, substantia nigra, pons, inferior olive, cerebellum, cortex, and autonomic nervous system. Unlike PD and DLB, in MSA α-syn is found predominantly within oligodendrocytes as glial cytoplasmic inclusions (GCIs; **Figure 1*e***), although many neuronal cytoplasmic inclusions composed of α-syn are also present (Spillantini et al. 1998). Intriguingly, it appears that neuronal inclusions predominate in regions of the brain that are relatively preserved (**Figure 1*d***). This suggests the possibility that this disease is initiated by α-syn dysfunction within neurons and that, as the disease progresses, residual oligodendrocytes develop cytoplasmic inclusions by engulfing α-syn deposits left behind by degenerating neurons. In any case, PD, DLB, and MSA together constitute a distinct class of neurodegenerative disorders collectively known as the synucleinopathies.

ENVIRONMENTAL AND GENETIC CAUSES OF PARKINSON'S DISEASE

The majority of PD cases are idiopathic. Many epidemiological studies have endeavored to identify environmental and genetic factors that contribute to the development of PD (see Elbaz & Moisan 2008 for a review). Concerning the environment, several studies have demonstrated an association between pesticide exposure and PD [odds ratio (OR) = 1.4–2.5] (Dick et al. 2007, Frigerio et al. 2006, Tanner et al. 2009). These chemicals are still alarmingly used as piscicides, insecticides, and herbicides. For example, rotenone, an organic compound derived from the roots of various plants, is used to kill nuisance fish and insects. Paraquat is used by farmers to eliminate weeds among crops. When administered to mice, rats, or primates, these agents elicit neurodegenerative phenotypes resembling PD (Betarbet et al. 2000, Norris et al. 2007, Sherer et al. 2003). Moreover, similar chemicals directly cause parkinsonism in humans. In the 1970s and 1980s, humans accidentally exposed to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a byproduct of the synthetic recreational opioid 1-methyl-4-phenyl-propionoxypiperidine (MPPP), developed instantaneous parkinsonism (Davis et al. 1979, Langston et al. 1983). Structurally, MPTP is quite similar to paraquat and is thought to have an identical mechanism of action. These chemicals exert toxicity by binding to mitochondrial complex I and interfering with oxidative phosphorylation (Ayala et al. 2007). The resulting mitochondrial dysfunction generates reactive oxygen species (ROS) that propagate toxicity through the oxidation of proteins, lipids, and nucleic acids (see Ayala et al. 2007 for a review). Thus, for the past three decades, much attention has been focused on mitochondrial dysfunction as the primary cause of PD.

Early studies associated manganese exposure with parkinsonism (Smyth et al. 1973), but recent studies have not corroborated this link (Stampfer 2009, Tanner et al. 2009). Indeed,

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miners exposed to high levels of manganese do develop a syndrome that clinically mimics PD (Mena et al. 1969). However, unlike PD, these individuals exhibit degeneration of the globus pallidus and striatum while the substantia nigra is spared (Perl & Olanow 2007). Furthermore, LBs do not form in these patients. Thus, manganism appears to be a parkinsonian disorder distinct from PD.

With respect to genetic factors relevant to PD, we have witnessed an explosion of information. In 1997, a missense mutation (A53T) in α-syn was identified in a family with a dominantly inherited form of PD (Polymeropoulos et al. 1997). Soon thereafter, it was recognized that α-syn was the major component of the LBs and LNs found in sporadic forms of the disease (Spillantini et al. 1997, 1998). In fact, the use of α-syn immunohistochemistry has substantially improved the recognition of cortical pathology that had previously been underappreciated in PD (Dickson et al. 2009). Additional mutations within α-syn (A30P, E46K) as well as duplication and triplication of the *SNCA* locus have subsequently been identified in families with dominant PD, further confirming the importance of this protein in the pathogenesis of the disease (Chartier-Harlin et al. 2004, Kruger et al. 1998, Singleton et al. 2003, Zarranz et al. 2004). Moreover, several recent genome-wide association studies have also associated α-syn polymorphisms with PD (Latourelle et al. 2009, Satake et al. 2009, Simon-Sanchez et al. 2008).

In addition to α-syn, at least six other genes have been associated with familial parkinsonism. Of these, mutations in *LRRK2* account for the largest percentage (>4%) of hereditary PD (Lees et al. 2009). *Parkin*, *PINK1*, and *DJ-1* mutations are associated with autosomal-recessive forms of parkinsonism and appear to have roles in sensing oxidative stress and regulating mitochondrial dynamics (Bonifati et al. 2003, Kitada et al. 1998, Valente et al. 2004); these results confirm the importance of preserving mitochondrial function in this disease. The identification of genetic risk factors that predispose individuals to develop idiopathic PD has proven to be more difficult (Lill et al. 2009). Despite the large number of population-based genome studies that have been conducted, the only genes with significant risk associations with PD are glucosylceramidase (*GBA*), *SNCA*, and *LRRK2* (Lill et al. 2009). GBA appears to be the most significant of these risk factors, with an OR of 2.31 (95% confidence interval = 1.61–3.32). GBA is mutated in the autosomal-recessive childhood disorder Gaucher disease, which features the accumulation of glucosylceramide within neurons and hepatocytes. Heterozygous individuals, who do not accumulate glucosylceramide within their neurons, are, however, at increased risk of developing PD with classical nigral degeneration and accumulation of α-syn in LBs and LNs (Neumann et al. 2009). This strong association of GBA with PD suggests that abnormalities in lipid metabolism also contribute to disease progression. These epidemiological, hereditary, and population-based studies clearly demonstrate that PD is a complex, multifaceted disorder in which α-syn toxicity, mitochondrial dysfunction, and abnormal lipid metabolism play central pathogenic roles.

BIOPHYSICAL PROPERTIES OF α-SYNUCLEIN

α-Syn was first isolated from the cholinergic neurons of *Torpedo californica* (Maroteaux et al. 1988). The protein localizes only to synaptic vesicles and portions of the nucleus—hence the name synuclein. Three additional synuclein family members have been identified and are named β-syn, γ-syn, and synoretnin (Lavedan 1998). β-Syn shares 90% homology in the N terminus and 33% homology in the C terminus with α-syn (Lucking & Brice 2000). γ-Syn and synoretnin are more divergent and share only 78% N-terminal and 6% C-terminal homology with α-syn (Lucking & Brice 2000). Only α- and β-syn are expressed in the mammalian brain (Lavedan 1998). All synucleins are characterized by a highly conserved imperfect sequence (KTKEGV) that is repeated ∼6 times throughout the N-terminal half of the protein (**Figure 2*a***; Maroteaux et al. 1988).

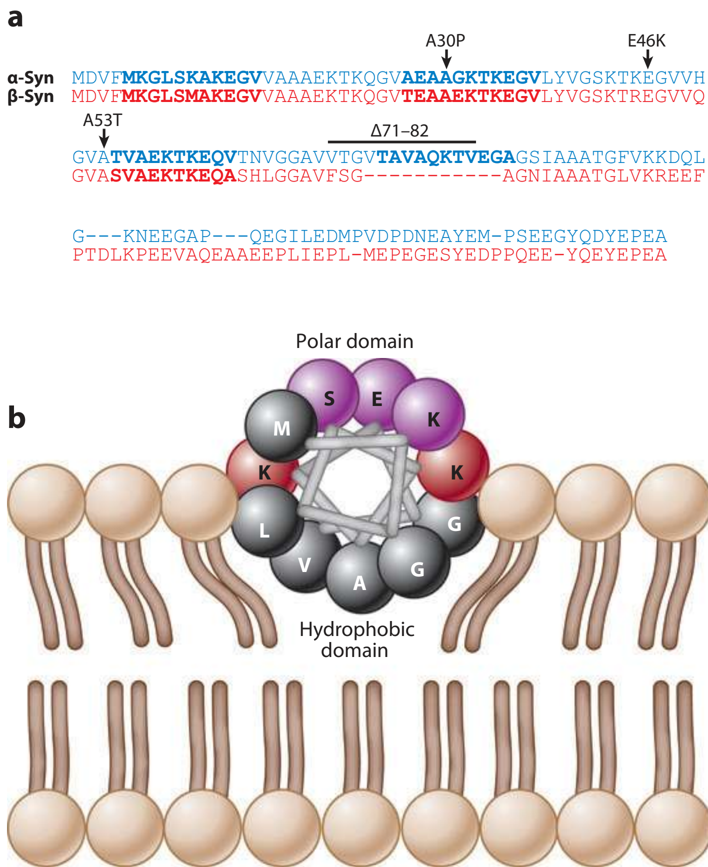

Figure 2

α-Synuclein (α-syn) and β-syn (*red* and *blue*, respectively) are highly homologous. (*a*) The highly conserved, imperfect repeats that form A2-type amphipathic α-helices are in bold. The hydrophobic core (Δ71–82), which is required for α-syn fibrillization in vitro and toxicity in vivo, is indicated by the horizontal line. This hydrophobic domain is absent in β-syn. Arrows identify the location of the point mutations associated with familial Parkinson's disease. (*b*) An axial view of α-syn residues when displayed on an Edmundson helix wheel. Polar residues (i.e., S, E, and K) face the hydrophilic environment of the cytosol, whereas hydrophophic residues are buried in the acyl chains of the phospholipid bilayer. Positively charged lysine residues (K) separate the polar and hydrophobic domains and interact directly with the anionic surface of the phospholipid bilayer.

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Despite the significant homology between α- and β-syn, only α-syn forms detergent-resistant amyloid deposits in PD, DLB, and MSA. The critical difference between α- and β-syn is the presence of a highly hydrophobic 12–amino acid stretch at its center (71-VTGVTAVAQKTV-82) (**Figure 2*a***; Giasson et al. 2001). This domain is required for the oligomerization and fibrilization of α-syn. Moreover, deletion or disruption of this domain through the addition of a charged amino acid abrogates α-syn's ability to form amyloid fibrils (Giasson et al. 2001). It is the amyloid form of α-syn that aggregates within neurons as LBs and LNs and within oligodendrocytes as GCIs. However, the body of evidence suggests that these α-syn amyloids are not required for toxicity (see below). Instead, early misfolded forms of α-syn, possibly dimers or small oligomers, are proposed to be the toxic species in the synucleinopathies.

The disease-associated mutations alter α-syn's structure in different ways. The A53T mutation effectively expands the hydrophobic domain from 11 amino acids to ~30 amino acids by destabilizing the α-helical domain between residues 51 and 66 (Biere et al. 2000). This expanded hydrophobic core confers gain-of-function toxicity (Biere et al. 2000) by facilitating the protein's ability to adopt the β-sheet structure required for the formation of oligomeric species (Conway et al. 1998, Giasson et al. 1999, Hashimoto et al. 1998). The A30P mutation disrupts the first α-helical domain of α-syn and reduces its affinity for phospholipids. This mutation also reduces amyloid formation in vitro (Yonetani et al. 2009), perhaps by shifting the equilibrium to more stable, soluble toxic oligomers. Finally, the E46K mutation is thought to elicit toxicity by altering α-syn's interactions with anionic phosopholipids, exposing the hydrophobic surfaces for potential intermolecular interactions (Rospigliosi et al. 2009). These increased intermolecular interactions would accelerate dimer formation and the subsequent generation of toxic oligomers.

α-SYNUCLEIN INTERACTS WITH LIPIDS AND MEMBRANES

Although the exact function of α-syn is still unclear, substantial evidence now exists to suggest that α-syn interacts directly with lipids and membranes both physiologically as well as pathologically. In solution, α-syn does not adopt a consistent secondary structure

(Weinreb et al. 1996) and is considered to be natively unfolded. However, in the presence of small diameter vesicles (20–25 nm) composed of acidic phospholipids, α-syn adopts an α-helical secondary structure (Davidson et al. 1998) that is ideally suited for lipid interactions. As shown in **Figure 2*b***, when the KTKEGV N-terminal repeats are displayed on an Edmundson helix wheel, they exhibit a distinct distribution of polar and nonpolar residues that form separate hydrophobic and polar faces reminiscent of A2-type amphipathic α-helices, motifs common to apolipoproteins and other lipid-binding proteins (Segrest et al. 1990, 1992). Only the N-terminal 102 residues partake in lipid binding (Perrin et al. 2000); the negatively charged C terminus remains disordered and is proposed to act as a scaffold to recruit additional proteins to the membranes (Eliezer et al. 2001).

The binding of α-syn to cholesterol- and sphingomyelin-containing vesicles could help in their stabilization, possibly protecting them from premature fusion as a result of curvature stress (Kamp & Beyer 2006). Indeed, when wild-type (WT) α-syn interacts with phospholipid micelles, it distorts and flattens their surface curvature (Perlmutter et al. 2009). A30P α-syn, with its decreased membrane affinity, does not alter the surface curvature of the micelles. Both A53T and E46K α-syn exhibited increased membrane-binding affinity and flattening of micelle surface curvature. For A53T α-syn, this is mediated through additional hydrogen bonding between threonine 53 and valine 49 in the backbone of α-syn (Perlmutter et al. 2009). For E46K α-syn, the charge replacement provided by lysine 46 increases hydrogen bonding between α-syn and the phospholipid surface (Perlmutter et al. 2009). These α-syn-induced membrane curvature changes can have a significant impact on the basal fusogenic properties of synaptic vesicles. Vesicles with high curvature favor fusion with flat target membranes to relieve the stress introduced by curvature (Chernomordik et al. 1995). By "flattening" the surfaces of vesicles, α-syn might relieve this stress and thus slow the fusion of vesicles with target membranes.

Interestingly, α-syn's association with lipid membranes also accelerates amyloid fibril formation (Jo et al. 2000). When WT, A30P, or A53T α-syn are incubated in the presence of synthetic vesicles containing polyunsaturated fatty acids (PUFAs), they form dimers and higher order oligomers (Perrin et al. 2001). Oligomerization does not occur in the presence of PUFAs alone or with vesicles containing saturated fatty acids. Notably, these oligomers are highly stable and resist disruption by prolonged boiling, high sodium dodecyl sulfate (SDS) concentrations, and treatment with urea (Perrin et al. 2001). Thus, it appears that α-syn's normal lipid interactions can precipitate the formation of dimers and toxic oligomers, perhaps by increasing the local concentration of α-syn.

α-SYNUCLEIN INTERACTS WITH MEMBRANES IN VIVO

α-Syn toxicity has been modeled in several organisms spanning the eukaryotic kingdom from yeast to mammals. Despite the evolutionary distances separating these model systems, they have all consistently revealed that endoplasmic reticulum (ER) stress, vesicle trafficking defects, impairment of the ubiquitin-proteasome system, and mitochondrial dysfunction result from α-syn overexpression. The yeast model, in particular, offers several experimental advantages that make it an ideal system to study human disease. First and foremost, the fundamental cellular processes governing metabolism, transcriptional regulation, cytoskeletal dynamics, organelle biogenesis, protein folding, trafficking, and secretion are highly conserved between yeast and mammalian cells. Second, the yeast genome is extremely well characterized and amenable to rapid genetic manipulation (Miller-Fleming et al. 2008, Outeiro & Giorgini 2006). Finally, the magnitude of already available yeast data relating transcriptional regulation to protein function allows for a comprehensive interrogation of the biological consequences of any biological perturbation (Hong et al. 2008).

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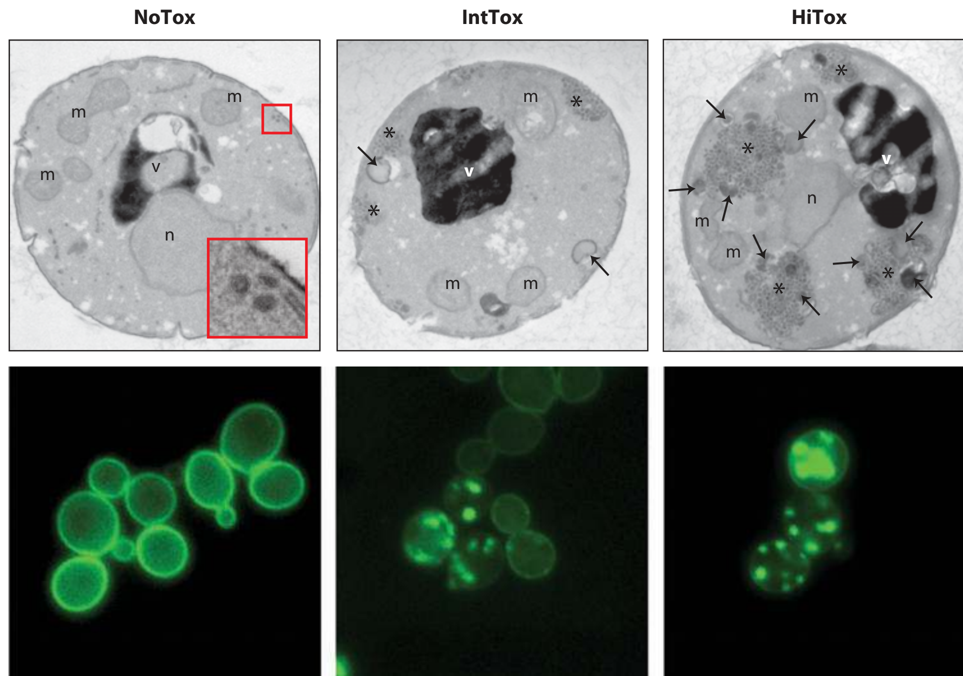
Exploiting these advantages, Outeiro & Lindquist (2003) developed a yeast model to study α-syn biology and pathobiology. WT as well as mutant (A53T or A30P) α-syn were placed under the control of a galactose-inducible promoter that allows for the synchronous induction of gene expression when the cells are transferred from glucose/raffinose to galactose media. At low expression levels, both WT and A53T α-syn localize evenly to the cell surface and do not alter the growth or viability of the yeast cells (**Figure 3**, NoTox). However, using electron microscopy (EM), a mild increase in the total number of secretory vesicles can be seen in the NoTox strain (**Figure 3**). At higher levels of expression, both WT and A53T α-syn redistributed from the cell surface into cytoplasmic foci (**Figure 3**, IntTox and HiTox) (Outeiro & Lindquist 2003), which were determined by immuno-EM to be collections of stalled vesicles decorated with α-syn (Gitler et al. 2008). These defects are accompanied by reduced growth and cytotoxicity (**Figure 3**). Further increasing the dosage of α-syn leads not only to an increase in toxicity but to the accumulation of cytoplasmic lipid droplets (Outeiro & Lindquist 2003), ER stress (Cooper et al. 2006), activation of the heat-shock response (Yeger-Lotem et al. 2009), and mitochondrial dysfunction (**Figure 3**; Su et al. 2010). This exquisite dosage dependency of toxicity and foci formation is reminiscent of the familial forms of PD caused by duplication or triplication of the *SNCA* locus (Chartier-Harlin et al. 2004, Ibanez et al. 2009, Singleton et al. 2003). Studies using this yeast α-syn model have the potential to truly expand our understanding of the human disease.

α-SYNUCLEIN CAUSES VESICLE TRAFFICKING DEFECTS

Recent investigations have begun to elucidate the mechanism(s) by which α-syn causes vesicles to accumulate. In healthy cells, vesicles bud from the ER and traffic to their target membranes, where they dock and fuse in a highly regulated fashion. Importantly, the same proteins involved in regulating these steps in yeast are highly conserved and closely related to their counterparts in other eukaryotes. In the presence of α-syn, vesicles efficiently bud from the ER but fail to dock and fuse with Golgi membranes (Gitler et al. 2008). In fact, this defect in ER-to-Golgi complex trafficking is one of the earliest abnormalities detectable in yeast cells expressing intermediately toxic levels of α-syn (Cooper et al. 2006). A more modest accumulation of vesicles also occurs in cells expressing nontoxic levels of α-syn, which suggests that vesicle accumulation is not simply a downstream consequence of α-syn toxicity. In a genetic screen designed to find modifiers of intermediate levels of α-syn toxicity (IntTox), the largest category of hits targets this step to either exacerbate or alleviate the block in ER-to-Golgi complex trafficking (Cooper et al. 2006). Among the strongest suppressors is the Rab GTPase Ypt1 (Rab1). Ypt1/Rab1 completely restores normal ER-to-Golgi complex trafficking in yeast cells expressing intermediate levels of α-syn and also restores α-syn localization to the plasma membrane. Rab GTPases play a key role in ensuring that vesicles both form correctly and are delivered to their appropriate target (Stenmark 2009). That these proteins constituted the largest class of suppressors indicates that the vesicle accumulation observed in yeast cells is intimately associated with toxicity. Moreover, the association between α-syn and defects in vesicle trafficking is not unique to yeast; it is also present in the worm, fly, and mammals.

α-Synuclein Induces Trafficking Defects in *Caenorhabditis elegans* and *Drosophila*

In *C. elegans*, PD has been modeled through the directed expression of α-syn in dopaminergic neurons (Lakso et al. 2003). These animals lose their dopaminergic neurons over a period of 72 hours. An early gene expression study of this worm model revealed significant upregulation of transcripts involved in



	NoTox	IntTox	HiTox
α-Syn localization	Membraneous	Membraneous Small foci	Large foci
Growth rate	Normal	Decreased	No growth
Vesicle accumulation	Mild	Moderate	High
ER-to-Golgi complex trafficking defect	Absent	Present	Present
Mitochondrial defects	None	Low	High
Lipid droplet accumulation	Absent	Rare	Present

Figure 3

The yeast model: three strains. α-Synuclein (α-syn) is tagged at the C terminus with green fluorescent protein (GFP) and expressed using the galactose-inducible promoter. At low copy number, α-syn is not toxic and localizes to the plasma membrane, which causes a mild increase in vesicle accumulation as seen by electron microscopy (EM) (NoTox strain). Increasing the dosage of α-syn results in toxicity and the redistribution of α-syn from the surface of the yeast cell to cytoplasmic foci (IntTox strain). At extremely high levels of α-syn expression, yeast cells accumulate cytoplasmic lipid droplets and exhibit profound ER stress and mitochondrial dysfunction (HiTox strain). Arrow, lipid droplet; m, mitochondria; asterisk, stalled vesicle; v, vacuole; n, nucleus. NoTox micrographs are reprinted from Gitler et al. (2008), copyright © 2008, National Academy of Sciences, U.S.A.

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endocytosis, protein degradation, and respiration (Vartiainen et al. 2006). A separate small RNAi study also demonstrated that downregulation of the endocytic pathway increased the toxicity of α-syn (Kuwahara et al. 2008). Given the potent rescue of α-syn by Rab1 in yeast, the neuroprotective potential of various Rab proteins was tested in this worm model. In addition to Rab1, which promotes ER-to-Golgi complex flux, other Rabs known to act on the *trans*-Golgi network (Rab8) also alleviate the toxicity of α-syn to dopaminergic neurons in worms (Cooper et al. 2006, Gitler et al. 2008), which suggests that α-syn causes defects in multiple vesicle trafficking steps.

In fruit flies, overexpression of the human α-syn transgene results in age-dependent dopaminergic neuron loss and the formation of ubiquitinated cytoplasmic protein inclusions reminiscient of LBs and LNs (Feany & Bender 2000). Although α-syn's interactions with lipids and membrane trafficking has not been directly examined in *Drosophila*, it appears that defects in vesicle trafficking contribute to neurotoxicity in this model as well. In *Drosophila*, the Rab1 GTPase mitigates the toxicity of WT and A53T α-syn just as it does in yeast (Cooper et al. 2006), which implies that α-syn-induced neurotoxicity in the fly is at least partially caused by blocking vesicle trafficking pathways. Transcriptional profiling of α-syn-expressing flies at presymptomatic (1-day posteclosion) and diseased (10- and 30-days posteclosion) stages has also revealed alterations in lipid metabolism (Scherzer et al. 2003). Specifically, the differentially expressed transcripts were enriched for genes regulating catecholamine synthesis, mitochondrial function, and lipid binding (Scherzer et al. 2003). One of the downregulated transcripts, phospholipase A2, is postulated to facilitate the exocytosis of neurotransmitters at synapses through the generation of specific lysophospholipids (Davletov et al. 2007), phospholipids that have had one of their fatty acid chains removed. When inserted into lipid bilayers, lysophospholipids allow the surface to kink and bend, forming "dimples" that attract synaptic vesicles (Davletov et al. 2007). Thus, the downregulation of phospholipase A2 may be a compensatory response to α-syn expression, the details of which remain to be determined.

α-Synuclein Regulates the Distal Reserve Pool of Synaptic Vesicles

Mammalian systems have also demonstrated defects in vesicle trafficking as a consequence of α-syn overexpression. In rat midbrain neuronal cultures, lentiviral expression of α-syn yields neurotoxicity with preferential dopaminergic toxicity (Cooper et al. 2006). Overexpression of human Rab1 reduces the toxicity of α-syn in this system as well (Cooper et al. 2006). Recent studies in HEK and PC12 cells have shown that overexpression of α-syn elicits an ER-to-Golgi complex trafficking block that is nearly identical to the trafficking defect seen in yeast (Thayanidhi et al. 2010). In the presence of α-syn, COPII-coated vesicles bud from the ER but fail to fuse with their target Golgi membranes (Thayanidhi et al. 2010). Normal trafficking can be restored by the co-overexpression of ykt6, a v-SNARE that promotes vesicle fusion and also has been identified as a suppressor of α-syn toxicity in yeast (Cooper et al. 2006).

Abnormal vesicle trafficking has also been linked to α-syn in vivo in mice. Targeted deletion suggests that α-syn normally acts to regulate the pool of vesicles available at the synapse. Mice lacking α-syn exhibit defects in paired-pulse inhibition of dopaminergic nerve terminals within the striatum (Abeliovich et al. 2000). In other words, dopaminergic synapses inappropriately release excessive quantities of neurotransmitter in response to paired stimuli, implying that the mechanisms that limit the number of vesicles released are defective. Indeed, this defect is accompanied by an overall reduction in size of the distal reserve pool of synaptic vesicles (Cabin et al. 2002). In hippocampal slice cultures, antisense RNA knockdown of α-syn similarly yields a 50% reduction in the distal reserve synaptic vesicle pool with no alteration

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in the numbers of vesicles docked at the synapse (Murphy et al. 2000). Conversely, modest overexpression of α-syn in hippocampal neurons is associated with a reduction in the number of docked vesicles, which leads to deficits in the release of neurotransmitters while increasing the number of vesicles available in the distal reserve pool (Nemani et al. 2010). Thus, it appears that α-syn normally assists in the regulation and/or maintenance of vesicle stores at the presynaptic membrane.

However, too much α-syn results in gain-of-function toxicity. In mice, overexpression of α-syn with the PrP promoter results in a tenfold increase in α-syn protein levels in the spinal cord and only a three to fourfold increase in the cerebral cortex. This high level of transgene expression elicits profound motor neuron degeneration accompanied by debilitating motor deficits (Giasson et al. 2002, Gomez-Isla et al. 2003, Lee et al. 2002). These mice simply do not survive long enough to develop cortical or brainstem pathology. Ultrastructural examination of motor neurons in these mice reveals mitochondria, vacuoles, and stalled vesicles trapped within degenerating axons (Giasson et al. 2002). In fact, immunohistochemical studies of PD brains have demonstrated that the synaptic vesicle proteins synaptophysin and chromogranin A colocalize to the periphery of LBs (Nishimura et al. 1994) where, as shown by EM, numerous small diameter (20–100 nm) vesicles are embedded (Soper et al. 2008). Thus, under abnormally nontoxic conditions, it appears that α-syn may still interact with membranes and vesicles, but in ways that promote their dysfunction.

Finally, in a similar PrP-A30P α-syn mouse model, it was demonstrated that the mutant form of α-syn coimmunoprecipitated with Rab GTPases corresponding to synaptic vesicles (Rab 3A), endosomes (Rab5), and the *trans*-Golgi network (Rab8) (Dalfo et al. 2004). A role for Rab proteins in PD is further supported by rat studies using the dopaminergic neurotoxin 6-hydroxydopamine (6-OHDA). In these experiments, overexpression of Rab3b protects dopaminergic neurons from the toxicity of 6-OHDA by increasing the number and size of synaptic vesicles (Chung et al. 2009). These findings suggest that α-syn might directly interact or interfere with Rab proteins on membrane surfaces, thereby impairing the proper trafficking of vesicles to their target membranes.

The sum of this data from yeast through humans consistently demonstrates that α-syn normally assists in maintaining pools of synaptic vesicles available for release. However, alteration of α-syn's expression levels or biophysical properties results in vesicle trafficking abnormalities within a variety of cellular compartments including the ER and Golgi complex as well as at the synapse.

α-SYNUCLEIN CAUSES MITOCHONDRIAL DEFECTS

Microarray studies with yeast expressing high levels of α-syn (HiTox) reveal that mitochondrial dysfunction and oxidative stress are also significant features of α-syn toxicity (Flower et al. 2005, Su et al. 2010). The presence of mitochondrial dysfunction is noteworthy given the evidence already implicating mitochondrial toxins in the pathogenesis of PD. Moreover, it also demonstrates that the complex, multifaceted pathophysiology of PD is recapitulated in this simple multicellular organism. In these HiTox α-syn yeast cells, mitochondria fragment, swell, and produce ROS (**Figure 3**, HiTox). This is in addition to the vesicle trafficking defects already noted in the IntTox strain. To explore the toxic connections between vesicle trafficking and mitochondrial dysfunction, a high-throughput chemical screen was carried out; it identified four closely related compounds (1,2,3,4-tetrahydroquinolinones) that significantly attenuated toxicity (Su et al. 2010). These compounds reverse the accumulation of α-syn cytoplasmic foci, which restores normal ER-to-Golgi complex trafficking and alleviate the mitochondrial dysfunction associated with the HiTox α-syn yeast strain (Su et al. 2010). These compounds also protect against α-syn

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toxicity in *C. elegans* and rat midbrain neuronal cultures.

The pesticide rotenone is commonly used to model mitochondrial dysfunction in animal models (Cannon et al. 2009). In rat midbrain neuronal cultures, rotenone treatment results in preferential dopaminergic neuron toxicity that is similar to the toxicity associated with α-syn expression in these cells. Notably, the compounds identified in the high-throughput chemical screen, which were selected for their ability to rescue α-syn toxicity in yeast, also protect against rotenone toxicity in rat midbrain neurons (Su et al. 2010). Therefore, these compounds must be acting on some deeply rooted biological process linking α-syn toxicity, vesicle trafficking defects, and mitochondrial dysfunction.

Mitochondrial dysfunction and the production of ROS is also a consistent feature of models of other PD-associated genes, namely *parkin*, *PINK1*, and *DJ-1* (Clark et al. 2006, Heo et al. 2010, Meulener et al. 2006, Pesah et al. 2004, Piccoli et al. 2008, Yang et al. 2005). Loss-of-function mutations in *parkin* and *PINK1* produce abnormalities in mitochondrial fission, fusion, and mitophagy. Normal mitochondrial dynamics is required to maintain the health of mitochondria and allows for the removal or damaged organelles. Defects in these processes produce ROS that can elicit toxicity directly through the oxidation of nucleic acids and lipids or indirectly by promoting the misfolding and oligomerization of α-syn (Norris et al. 2003). *DJ-1* loss-of-function mutations are associated with a defective cellular response to ROS (Meulener et al. 2006). Finally, manganism is a form of parkinsonism associated with manganese toxicity. Manganese is thought to elicit ROS production by directly interfering with oxidative phosphorylation (Brown & Taylor 1999). Remarkably, the yeast genetic screen for modifiers of α-syn toxicity, which included 60 metal ion transporters, only identified three, all of which regulated manganese concentrations (Yeger-Lotem et al. 2009). The first, Ccc1, regulates the transport of both manganese and iron. The second, Pmr1, transports calcium and manganese. The third was a protein of unknown function (now called Ypk9) that turned out to be a manganese transporter that strongly suppressed α-syn toxicity (Gitler et al. 2009). Moreover, Ypk9 is the yeast homolog of ATP13A2/PARK9, which is associated with Kufor-Rakeb syndrome, an aggressive parkinsonian disorder with dementia. WT Ypk9 also potently restores the localization of α-syn to the plasma membrane, thus providing additional indirect evidence linking ER-to-Golgi complex trafficking defects with mitochondrial dysfunction.

ResponseNet REVEALS HIDDEN α-SYNUCLEIN-INDUCED ABNORMALITIES IN LIPID/STEROL METABOLISM AND THE STRESS RESPONSE

Side-by-side comparison of data from the yeast genetic screen with the transcriptional profiles of yeast expressing α-syn reveals little overlap between the two data sets (Yeger-Lotem et al. 2009). In fact, the data sets almost appear to be mutually exclusive. This situation is not unique to α-syn or to yeast and has been noted in several different systems. Genetic screens, by their nature, identify transcriptional regulators with the capacity to significantly up- or downregulate entire pathways or processes. In contrast, RNA profiles reflect the consequences of a specific perturbation. Thus, these two methodologies interrogate different aspects of disease biology. To bridge this information gap, Yeger-Lotem, Riva, and colleagues developed an algorithm, ResponseNet, that connected the genetic hits to the transcriptional responses (Yeger-Lotem et al. 2009). Development of this algorithm was possible only because a wide and thorough interactome for virtually the entire yeast proteome is publically available (Hong et al. 2008). Applying this algorithm to the yeast α-syn data sets identified ergosterol biosynthesis, the stress response, and the target of rapamycin (TOR) pathway as additional cellular processes critical to α-syn toxicity (Yeger-Lotem et al. 2009).

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ResponseNet: α-Synuclein and Lipid/Sterol Metabolism

Lipid and sterol biosynthetic pathways have a complex relationship with PD. Several early studies demonstrated alterations in lipid content in PD brains. Biochemical analyses of brains from PD patients reveal decreased levels of phosphatidylethanolamine, phosphotidylcholine (Riekkinen et al. 1975, Van Woert & Mueller 1971), and PUFAs (Dexter et al. 1994). In contrast, levels of sphingomyelin are elevated in PD brains (den Jager 1969). Alterations in sphingolipid metabolism could help explain why individuals with heterozygous GBA mutations do not develop Gaucher disease and do not accumulate glucosylceramide within their neurons, but instead are at increased risk for developing classical PD (Neumann et al. 2009, Wong et al. 2004). Intriguingly, the catabolism of sphingolipids, including glucosylceramide, produces sphingosine, which facilitates synaptic vesicle exocytosis (Darios et al. 2009). Perhaps subtle abnormalities in sphingolipid biosynthesis and metabolism impair vesicle trafficking at the synapse, focally increasing the local concentration of α-syn and thus precipitating a cascade of toxic events that results in the development of PD.

In yeast, expression of α-syn also results in abnormal lipid metabolism including the accumulation of cytoplasmic lipid droplets (**Figure 3**; Outeiro & Lindquist 2003, Su et al. 2010). Analysis of the ResponseNet results suggested that these lipid abnormalities might be due to defects in the mevalonate-ergosterol pathways (Yeger-Lotem et al. 2009). The ResponseNet algorithm highly ranked the genetic hit Hrd1, a ubiquitin protein ligase that targets 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase, and a genetic intermediary, Hap1, a transcriptional regulator of Erg11, which catalyzes ergosterol biosynthesis. To directly test the consequences of inhibiting ergosterol biosynthesis, α-syn yeast were grown in the presence of lovastatin, an inhibitor of HMG-CoA reductase, the rate-limiting enzyme in cholesterol/ergosterol biosynthesis. Lovastatin enhanced the toxicity of α-syn, whereas ubiquinone, a product of HMG-CoA reductase, provided modest suppression of α-syn toxicity (Yeger-Lotem et al. 2009). Strikingly, a similar effect of lovastatin has been described in humans. In a case report, treatment with lovastatin resulted in the precipitous development of parkinsonian symptoms, which were reversed upon cessation of the drug (Muller et al. 1995). Intriguingly, individuals with PD often have significantly lower levels of low-density lipoprotein (LDL) cholesterol than their spouses, possibly owing to reduced HMG-CoA reductase activity (Huang et al. 2008). However, a retrospective study of >700,000 elderly individuals found that treatment with simvastatin, but not lovastatin, delayed the progression to dementia in the PD cohort (Wolozin et al. 2007). Nonetheless, in this study the individuals who received simvastatin were actually being treated for elevated levels of cholesterol. The generally low cholesterol levels in PD patients might directly contribute to the development of the disease. Because cholesterols are critical for membrane biogenesis (Nohturfft & Zhang 2009), decreased cholesterol biosynthesis has the potential for profound toxic synergy with α-syn-induced vesicle trafficking defects.

ResponseNet: α-Synuclein and the Stress Response

ResponseNet also predicted the involvement of two key regulators of the stress response: heat shock protein 90 (Hsp90) and heat shock factor 1 (Hsf1) (Yeger-Lotem et al. 2009). Modifiers of the stress response, which are critical regulators of protein folding, were otherwise conspicuously absent in both the genetic and transcriptional data sets. However, it had already been demonstrated that genetic or chemical enhancement of the stress response potently protects against α-syn toxicity in *Drosophila* (Auluck & Bonini 2002, Auluck et al. 2002). Overexpression of Hsp70 or treatment with geldanamycin, a pharmacological potentiator of the stress response, fully prevent dopaminergic neuron loss in flies expressing α-syn. However, contrary to expectations, activation

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of the stress response increased the amount of detergent-insoluble α-syn while rescuing toxicity (Auluck et al. 2005). This finding is consistent with the hypothesis that the large, insoluble forms of α-syn are in fact not toxic and that other forms of α-syn (dimers, oligomers) account for toxicity.

Similarly, in yeast, Hsp70 overexpression or treatment with geldanamycin reduces toxicity, restores α-syn localization to the membrane, and reduces the generation of ROS (Flower et al. 2005). Also in yeast, α-syn toxicity is evidently not associated with the formation of amyloid fibrils. Nevertheless, the same properties that α-syn requires to form fibrillar species in vitro are required for toxicity in yeast and *Drosophila*. Expression of an α-syn construct lacking the central hydrophic core [amino acids 71–82 (α-syn$^{\Delta 71-82}$)], which is required for self-assembly in vitro, localizes to the plasma membrane of yeast just like the WT protein (Soper et al. 2008). However, unlike the WT protein, α-syn$^{\Delta 71-81}$ does not elicit the formation of α-syn-positive cytoplasmic foci or stalled vesicles and is not toxic in yeast (Soper et al. 2008) or in *Drosophila* (Periquet et al. 2007). Thus, membrane binding alone is insufficient for α-syn to elicit vesicle trafficking defects, mitochondrial dysfunction, and toxicity. A second step involving the central hydrophobic core is required for these consequent toxic events. It is possible that the formation of α-syn dimers and/or oligomers is this necessary toxic step.

MODEL OF α-SYNUCLEIN INTERACTIONS WITH LIPIDS AND MEMBRANES

In formulating a model to integrate the results of these diverse lines of investigation, we begin by considering the nature of the toxic α-syn species. Considerable biochemical evidence has demonstrated a propensity for α-syn to form amyloid fibrils, which can be found within the LBs, LNs, and GCIs of people suffering from PD or related synucleinopathies. Moreover, it appears that both fibril formation in vitro and toxicity in yeast and higher eukaryotes are mediated by the same biophysical properties of α-syn. Specifically, fibril formation is dependent on the 12 amino acids (71–82) that constitute the central hydrophobic core of the protein. Disruption of this domain abrogates the toxic potential of α-syn without interfering with its ability to interact with lipids. Strikingly, however, although α-syn protein aggregates form in *Drosophila* and mouse models, these α-syn amyloids are not required for toxicity. Furthermore, the aggregates do not form in the yeast model, where α-syn is toxic. Instead, dimers or small oligomers are proposed to be the toxic species in the synucleinopathies. We posit that these early misfolded forms of α-syn initially form through interactions with lipids and membranes.

Under normal conditions, α-syn exists briefly as a natively unfolded protein within the cytoplasm (**Figure 4*a***). In the presence of lipids and membranes, α-syn adopts a folded α-helical conformation that specifically favors binding to the anionic phospholipid surfaces of vesicles (**Figure 4*b***). At the synapse, transient interactions between dimeric α-syn molecules on the membrane surface or between α-syn molecules on neighboring vesicles help to hold these vesicles in a reserve pool distal from the synapse, thereby helping to regulate the number of synaptic vesicles docked at the synapse (**Figure 5*a***). This effect might simply be mediated by α-syn's direct interactions with vesicle membranes, by effects on membrane curvature, or by regulating the formation of membrane microdomains important for the recruitment of Rab proteins. Any alteration in Rab protein localization could affect the targeting of vesicles between organelles. In the absence of α-syn, or with the A30P mutation, which does not interact with phospholipid membranes, this reserve pool would be depleted, as these vesicles would either degrade or fuse too efficiently with their target membranes (**Figure 5*c***). Conversely, elevated expression levels of WT, A53T, or E46K α-syn, which do interact with phospholipid membranes, would strengthen these transient interactions between α-syn and itself, lipids, or other proteins and result in vesicle

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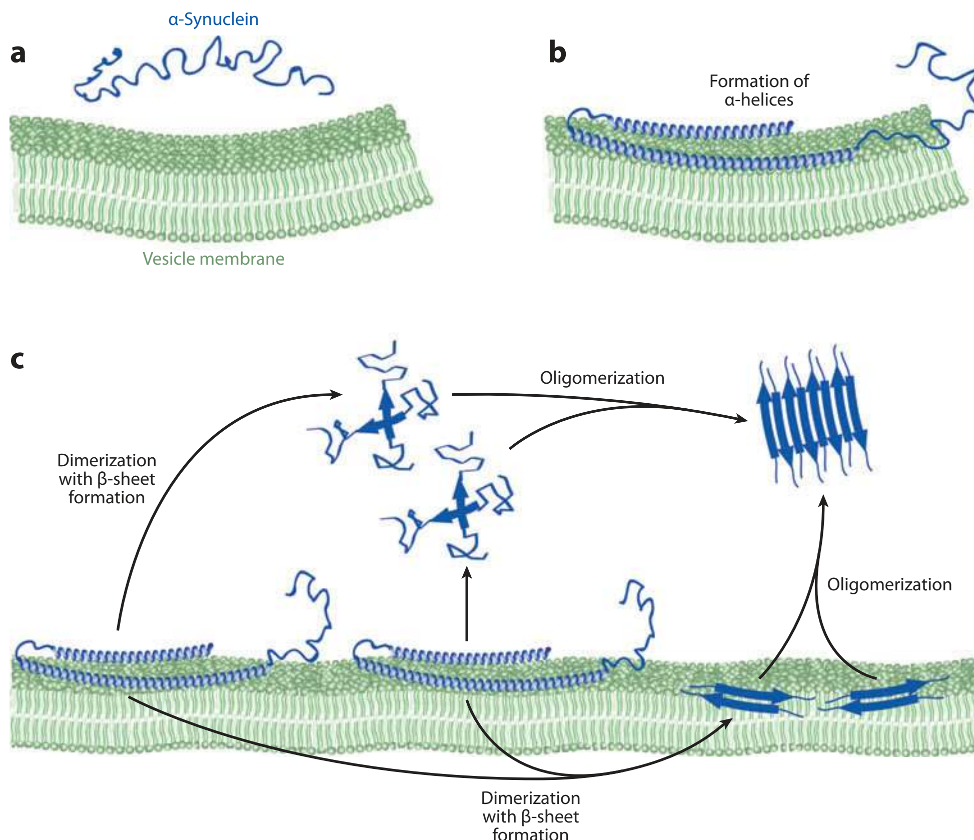

Figure 4

α-Synuclein (α-syn) requires lipid interactions to form dimers, oligomers, and mature amyloid fibrils. (*a*) In solution, α-syn is natively unfolded. (*b*) In the presence of vesicle membranes containing anionic phospholipids, the N terminus of α-syn forms two α-helices that allow it to associate with the surface of the membrane. (*c*) Stabilization of α-syn's membrane interactions (through the A53T or E46K mutations) or increased α-syn concentration (through genetic multiplication) facilitates the formation of α-syn dimers on the membrane surface or in the cytoplasm. Through dimerization, α-syn adopts a β-sheet secondary structure that, through association with α-syn monomers or other dimers, leads to oligomer formation. These oligomers seed fibril formation and deposit as amyloid within Lewy bodies and Lewy neurites.

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accumulation (**Figure 5*b***). The increased local concentration of α-syn on these stalled vesicles favors the nucleation of higher-order oligomeric species (**Figure 4*c***). These oligomeric species could then be free to disperse and interfere with trafficking between other compartments within the neuron. Ultimately, these oligomers may come together to form the amyloid fibrils (**Figure 4*c***) found in LNs and LBs, which in fact may simply be an inert epiphenomenon.

CONSEQUENCES OF α-SYNUCLEIN LIPID AND MEMBRANE INTERACTIONS

How might α-syn's abnormal lipid interactions mediate the diverse pathological features seen in PD? Abnormal interactions between α-syn and vesicles likely are initiated at the synapse, where α-syn is normally located (**Figure 6**). However, under pathological conditions, α-syn may also interact with and disrupt vesicle trafficking in other compartments where it

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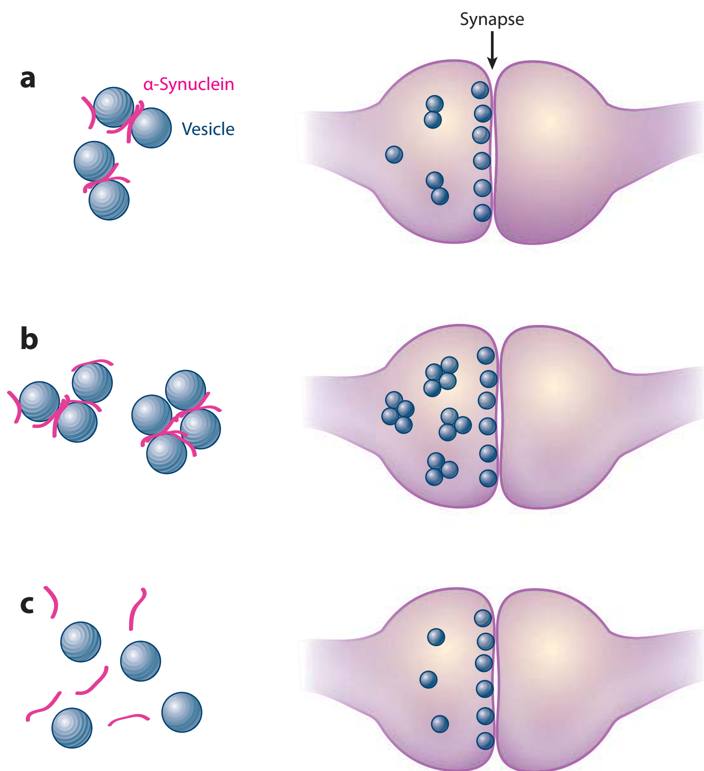

Figure 5

α-Synuclein (α-syn) modulates vesicle pools at the synapse and in other cellular compartments. (*a*) In a healthy neuron, α-syn localizes to the synaptic vesicles, which through direct effects and interactions with neighboring α-syn molecules helps to retain these vesicles in a distal reserve pool. (*b*) Overexpression of wild-type, A53T, and/or E46K α-syn enhances lipid interactions and alters the physical properties of these vesicles (i.e., reduces curvature), which leads to increased clustering of vesicles in the reserve pool. (*c*) With the A30P mutation (or in the absence of α-syn), α-syn is unable to bind lipid membranes and cannot act to retain vesicles in the distal reserve pool.

typically is not found. Within the ER, α-syn likely acts to inhibit ER-to-Golgi complex trafficking. The stalling of vesicles within this compartment alone can have several pathological consequences, particularly upon mitochondria. For example, a disruption of normal ER homeostasis might interrupt the autophagic destruction of damaged mitochondria (mitophagy). A failure in mitophagy would allow dysfunctional mitochondria to accumulate, thereby exacerbating cellular toxicity through the generation of ROS and impaired metabolism (**Figure 6**).

α-Syn-mediated ER dysfunction might also interfere indirectly with connections between the ER and mitochondria. In yeast (Kornmann et al. 2009) and mammalian cells (de Brito & Scorrano 2008), focal attachments between mitochondria and the ER allow mitochondria to sense and take up Ca^{2+} released by the ER into the cytosol. Additionally, these ER-mitochondrial tethers allow the two organelles to coordinate lipid synthesis (Daum & Vance 1997, Kornmann et al. 2009). Phosphatidylserine synthesized in the ER is transferred to mitochondria, where it is converted to phosphatidylethanolamine by Psd1 and then shuttled back to the ER for subsequent modifications to form phosphatidylcholine (Kornmann et al. 2009). α-Syn might disrupt this biosynthetic pathway by disrupting the flow of components between these organelles, resulting in the deficient sythesis of phosphatidylcholine. Indeed, untreated PD patients exhibit lower-than-normal levels of both phosphatidylethanoamine and phosphatidylcholine (Riekkinen et al. 1975).

Abnormal interactions between α-syn and the membrane at the synapse also provide an explanation for why catecholaminergic nuclei might be affected earlier than other neuronal subtypes in PD. Norepinephrine and dopamine are the catecholaminergic neurotransmitters found in the locus ceruleus and substantia nigra, respectively. Both are synthesized in the cytoplasm, where they are rapidly packaged into synaptic vesicles by vesicle momoamine transporter 2 (VMAT2) (see Caudle et al. 2008 for a review). Cytoplasmic retention of catecholamines yields cytotoxicity through the generation of oxidized dopamine adducts and ROS (Caudle et al. 2008, Mosharov et al. 2009). α-Syn, through the disruption of normal vesicle cycling at the synapse, could cause an excess of cytosolic catecholamines that would make these neurons particularly sensitive to additional cytotoxic stressors elicited by α-syn (**Figure 6**).

POTENTIAL FOR THERAPEUTIC INTERVENTION

The mainstay treatment for PD is the elevation of dopamine levels through the

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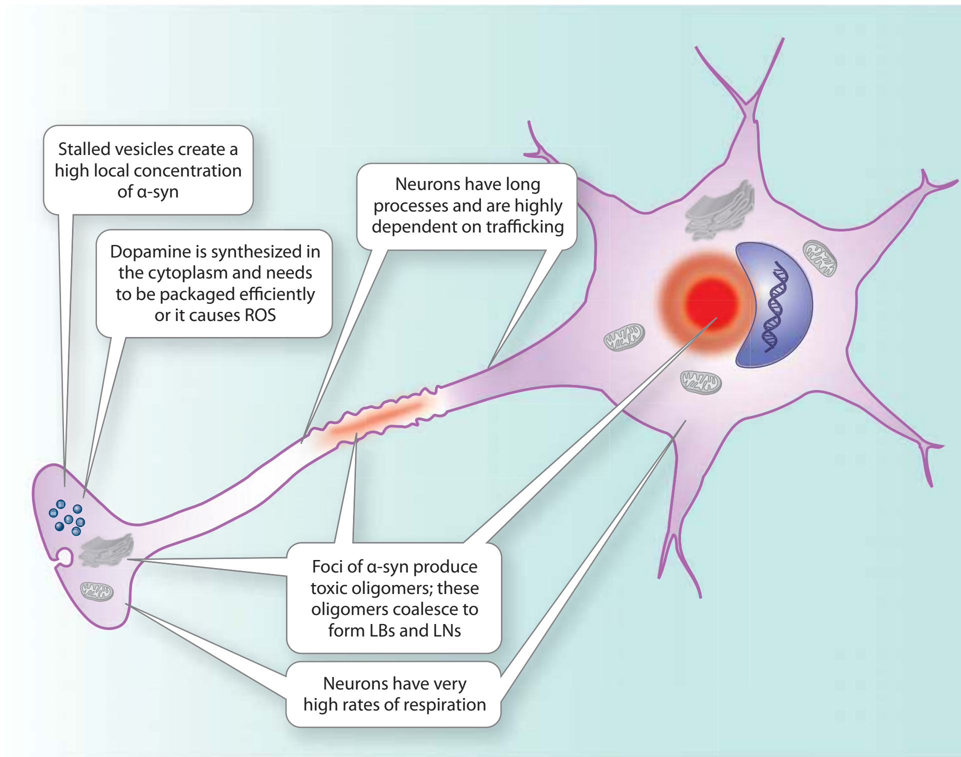

Figure 6

Summary schematic of α-synuclein toxicity in a dopaminergic neuron. LBs, Lewy bodies; LNs, Lewy neurites; ROS, reactive oxygen species.

administration of dopamine-receptor agonists, levodopa, or both. However, these agents do not address the underlying cell biological defects, and the disease progresses despite treatment. Therefore, much attention has been focused on the development of new therapeutic strategies. α-Syn's propensity to misfold and accumulate as cytoplasmic aggregates led to the hypothesis that toxicity could be prevented by interfering with the process of misfolding and aggregation (Beyer & Ariza 2008). In fact, a peptide ligand in silico screen against the central hydrophobic domain of α-syn has yielded compounds capable of inhibiting α-syn fibrillization (Abe et al. 2007). However, the utility of these compounds remains to be tested in vivo, and unless they address the early steps of α-syn oligomerization, it is questionable whether this approach will be therapeutically feasible.

This α-syn misfolding hypothesis was also tested by the coexpression of heat-shock proteins with α-syn (Auluck et al. 2002, McLean et al. 2002) and by the adminstration of geldanamycin, which potentiates the stress response (Auluck & Bonini 2002, Flower et al. 2005). The stress response normally functions to ensure proper protein folding within the chaotic, crowded cellular environment. Remarkably, although activation of the stress response potently rescued α-syn toxicity in *Drosophila*, the aggregation of α-syn was not reversed (Auluck et al. 2002), and the amount of detergent-insoluble α-syn actually increased (Auluck et al. 2005). Thus, it was unclear exactly how the stress response was acting to protect against α-syn toxicity, but it appeared that chaperones might protect against toxicity by accelerating the fibrillization of α-syn oligomers.

In yeast, where α-syn does not form fibrillar species, modulation of the stress response reduces the formation of α-syn foci, presumably by assisting vesicle trafficking pathways (Flower et al. 2005). It is possible that chaperones could do both. It has recently been shown that Hsp90 binds to α-syn and promotes mature fibril formation (Falsone et al. 2009), thereby limiting the pool of toxic oligomers available to interact with membranes. Additionally, heat-shock proteins can act on vesicle trafficking pathways directly. Both Hsp90 and Hsp70 promote flux from the ER to the Golgi complex, assist in vesicle fusion, and facilitate the retrieval of Rabs to their target membranes (Chen & Balch 2006, Sakisaka et al. 2002). Although modulation of the stress response appears to be an attractive target, this therapeutic strategy is complicated by the extensive involvement of heat-shock proteins in numerous cellular pathways (see Whitesell & Lindquist 2005 for a review). It has also been shown that the stress response facilitates the development of tumors in mice (Dai et al. 2007). Thus, although it may be possible to titrate chaperone modulators such that only stressed cells are affected, a more targeted approach might be preferable.

We propose that specifically targeting α-syn's abnormal interactions with membranes, or their immediate consequences, could have therapeutic potential in PD. The yeast chemical screen described above identified a class of compounds capable of alleviating the ER-to-Golgi complex trafficking defects and mitochondrial dysfunction associated with α-syn toxicity (Su et al. 2010). These compounds were also effective in neuronal models of α-syn and rotenone toxicity. This screen demonstrates that it is possible to develop drugs targeting α-syn's interactions with lipids and vesicle trafficking to create novel therapeutic strategies that specifically act on this upstream toxic event. Because α-syn toxicity appears to be initiated on membrane surfaces, compounds interfering with this interaction could have profound biological and clinical implications. By reducing ROS, restoring normal vesicle trafficking, and normalizing lipid/sterol metabolism, it may be possible to arrest the progression of PD.

CONCLUSIONS

Simple model organisms such as yeast have proven to be powerful tools in elucidating the cellular toxicity mechanisms underlying complex human diseases such as PD. These yeast studies have revealed central aspects of the biology and pathobiology of α-syn, implicating defects in ER-to-Golgi complex trafficking, mitochondrial function, the stress response, and lipid/sterol biosynthesis. Moreover, these findings complement studies in *C. elegans*, *Drosophila*, and mammalian models of α-syn toxicity.

Here we present a simple model in which α-syn initiates toxicity at the synapse through abnormal membrane interactions (**Figure 6**). We posit that α-syn normally acts by altering the physical (curvature) properties of vesicles, thereby regulating vesicle fusion and maintaining the distal reserve pool of vesicles. Abnormal enhancement of this activity both at the synapse and in other cellular compartments would cause synaptic dysfunction and a profound ER-to-Golgi complex trafficking blockade.

The consequences of these defects could precipitate a cascade of toxic events in which membrane-bound α-syn dimers nucleate to form soluble oligomeric species that then disperse throughout the neuron to elicit additional damage to the ER, mitochondria, and other organelles. In parallel, the same properties that promote α-syn membrane interactions and oligomerization also result in the formation of detergent-insoluble amyloid fibrils of α-syn that accumulate within cell bodies and neurites as inert LBs and LNs. In short, α-syn normally acts as a critical regulator of synaptic vesicle dynamics in vertebrates, whereas an exaggeration of its membrane interactions leads to cellular dysfunction and the development of PD.

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DISCLOSURE STATEMENT

S.L. is a founder of, a former member of the Board of Directors, and has received consulting fees from FoldRx Pharmaceuticals, a company that investigates drugs to treat protein folding diseases. S.L. is an inventor on patents and patent applications that have been licensed to FoldRx. S.L. is also a member of the Board of Directors of Johnson & Johnson.

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