Cytosolic Proteins Regulate α-Synuclein Dissociation from Presynaptic Membranes*

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Intracellular accumulation of insoluble α-synuclein in Lewy bodies is a key neuropathological trait of Parkinson disease (PD). Neither the normal function of α-synuclein nor the biochemical mechanisms that cause its deposition are understood, although both are likely influenced by the interaction of α-synuclein with vesicular membranes, either for a physiological role in vesicular trafficking or as a pathological seeding mechanism that exacerbates the propensity of α-synuclein to self-assemble into fibrils. In addition to the α-helical form that is peripherally-attached to vesicles, a substantial portion of α-synuclein is freely diffusible in the cytoplasm. The mechanisms controlling α-synuclein exchange between these compartments are unknown and the possibility that chronic dysregulation of membrane-bound and soluble α-synuclein pools may contribute to Lewy body pathology led us to search for cellular factors that can regulate α-synuclein membrane interactions. Here we reveal that dissociation of membrane-bound α-synuclein is dependent on brain-specific cytosolic proteins and insensitive to calcium or metabolic energy. Two PD-linked mutations (A30P and A53T) significantly increase the cytosol-dependent α-synuclein off-rate but have no effect on cytosol-independent dissociation. These results reveal a novel mechanism by which cytosolic brain proteins modulate α-synuclein interactions with intracellular membranes. Importantly, our finding that α-synuclein dissociation is up-regulated by both familial PD mutations implicates cytosolic cofactors in disease pathogenesis and as molecular targets to influence α-synuclein aggregation.

α-Synuclein (α-syn) 3 is a member of a multigene synuclein family that is highly abundant in presynaptic terminals of mammalian brain (1, 2). The function of α-syn is poorly resolved, although it is attributed with wide ranging roles in vesicular trafficking and vesicle biogenesis and as a molecular chaperone (3). α-Syn is also implicated in a broad spectrum of neurodegenerative disorders collectively named synucleinopathies, being a primary component of Lewy bodies (4), and as a fragment in Alzheimer disease plaques (5). In a small number of pedigrees, autosomal-dominant inheritance of Parkinson disease (PD) is linked to either multiplication of the normal α-syn gene or to one of three missense mutations (A30P, E46K, and A53T) (6, 7).

In vitro studies suggest that α-syn is natively unfolded in aqueous solution, and exposure to lipids stabilizes the amino terminus in an amphipathic α-helix that aligns polar and nonpolar residues into opposing orientations (8–11). Presumably, this secondary structure confers the lipid-biding properties for direct membrane interaction such that purified recombinant α-syn can bind to small diameter artificial vesicles rich in acidic phospholipids (8, 12), to purified synaptic vesicles (13, 14), and to membranes within intact cells (15). Most studies examining α-syn membrane binding have used either artificial phospholipids or purified membranes, without consideration to the potential regulatory function of soluble or membrane factors that may be pertinent to disease progression. Previous investigations were equivocal on whether α-syn mutations (A30P and A53T) affect membrane interactions; the A30P or A53T mutation had little or no effect on α-syn binding (16, 17), the A53T mutation reduced (8) or increased (18, 19) the membrane binding, or the A30P had reduced binding to purified synaptic vesicles (14, 20, 21). Further complexity may also arise from reversible membrane interaction, akin to that of cytosolic regulators of vesicular trafficking whose direct or indirect association with vesicle membranes is necessarily transient (e.g. exocytosis: NSF, α/β-SNAP, rab3, rabphilin, synapsin, CAPS; endocytosis: clathrin/adaptor, dynamin) (22–24). Association and dissociation is coupled to the vesicle life cycle, dictated by cation fluxes, nucleotide triphosphate cleavage, and post-translational modifications. In the case of rab3, an additional mechanism governs its membrane attachment. Another cytosolic protein, GDP-dissociation inhibitor, is essential to extract rab3 off exocytosing vesicles and deliver it to newly synthesized vesicles (25–27).

It is unknown whether α-synuclein exchange occurs between soluble and membrane compartments or even whether α-syn can dissociate from reconstituted or biological membranes. The possibility that chronic dysregulation of subcellular α-synuclein pools may contribute to PD and other synucle-
Cytosol-regulated α-Synuclein Dissociation

A. Dissociation assay

Step 1: Synaptosomes and cytosol were prepared separately from brains of Tg mice expressing the human α-syn (WT, A30P, or A53T) and α-syn-deficient mice, respectively. Step 2: Hypotonically lysed synaptosomes, the washed membranes (α-syn donor) were co-incubated with α-syn−/− cytosol (α-syn acceptor). Step 3: Membrane and cytosolic fractions were separated by centrifugation and the supernatants were analyzed by Western blotting.

B. Acceptor and Donor fractions

Hypotonically lysed and washed

<table>
<thead>
<tr>
<th>KO cytosol</th>
<th>Isotonic buffer</th>
<th>Hypotonic buffer</th>
<th>10 min</th>
<th>20 min</th>
<th>30 min</th>
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<tr>
<td>α-synuclein</td>
<td>S</td>
<td>S</td>
<td>P</td>
<td>S</td>
<td>P</td>
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<tr>
<td>GAPDH</td>
<td>S</td>
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inopathies prompted our search for cellular factors that affect α-synuclein membrane interactions. To address this, we developed an assay comprised of permeabilized synaptosomes and report here that α-syn dissociation from presynaptic membranes requires cytosolic proteins from the brain. Particularly relevant to disease pathogenesis was the finding that cytosol-dependent α-syn dissociation was significantly increased by two PD-related mutations (A30P and A53T) suggesting a mechanism whereby cytosolic cofactors can regulate α-syn solubility and, thereby, modulate disease progression.

EXPERIMENTAL PROCEDURES

Transgene Construction and Generation of Transgenic Mice—The human α-syn gene containing a eukaryotic Kozak initiation sequence (GCG GCC ACC) (28, 29) upstream of the start codon was ligated into a linearized cos-Tet expression vector containing the Syrian hamster prion protein promoter gene (30, 31). A30P and A53T α-syn mutant transgenes were generated using the Quick change kit (Stratagene). Following microinjection into fertilized oocytes of FVB/N mice, positive founders were identified by amplification of genomic DNA and bred to FVB wild-type mice. To generate α-syn Tg mice lacking endogenous murine α-syn, the α-syn Tg mice were crossed with SNCA−/− mice (Jackson Laboratories). F1 offspring positive for α-syn transgene were backcrossed and F2 progeny were selected for the presence of the neomycin resistance gene and the absence of the endogenous α-syn gene as described previously (32). All animal experiments were performed according to guidelines established in the Canadian Guide for the Care and Use of Laboratory Animals.

Synaptosome Preparation—Synaptosomes were prepared as described (26, 33). Briefly, brains from mice (A30P, A53T, and Wt transgenic mice) were dissected and homogenized with 10 strokes at 500 rpm, in ice-cold buffer A (320 mM sucrose, 1 mM EGTA, and 5 mM HEPES (pH 7.4)) and centrifuged at 3000 × g for 10 min. The supernatant was spun for 10 min at 14,000 × g and the pellet (P2) resuspended in buffer A. The P2 fraction was loaded onto a discontinuous Ficoll gradient (13, 9, and 5% in buffer A) and centrifuged for 35 min at 35,000 × g. The 13–9% interface, containing intact synaptosomes, was resuspended in buffer B (140 mM NaCl, 5 mM KCl, 20 mM HEPES, 5 mM NaHCO3, 1.2 mM Na2HPO4, 1 mM MgCl2, 1 mM EGTA, and 10 mM glucose) and spun at 14,000 × g for 10 min. The pellet was hypotonically lysed by two washes in buffer C (10 mM HEPES, 18 mM KOAc, 125 mM KOAc, and 2.5 mM MgCl2). After centrifugation (14,000 × g for 10 min), synaptosomes were resuspended in buffer D and incubated (as indicated in each figure legend) with or without α-syn deficient brain or liver cytosol before separating membrane and supernatant by centrifugation at 14,000 × g for 10 min. The supernatant was centrifuged at 100,000 × g to remove trace contaminating elements such as broken membranes or dissociated synaptic vesicles before quantifying soluble α-syn by Western blotting.

Cytosol Preparation from α-Syn Knock-out and Non-transgenic Mice—Mouse brains were homogenized in 10 volumes of homogenizing buffer (1 mM sucrose, 1 mM KOAc, 100 mM MgOAc, 1 mM HEPES (pH 7.4)). The homogenate was spun at

NaHCO3, 1.2 mM Na2HPO4, 1 mM MgCl2, 1 mM EGTA, and 10 mM glucose) and spun at 14,000 × g for 10 min. The pellet was hypotonically lysed by two washes in buffer C (10 mM HEPES, 18 mM KOAc, 125 mM KOAc, and 2.5 mM MgCl2). After centrifugation (14,000 × g for 10 min), synaptosomes were resuspended in buffer D and incubated (as indicated in each figure legend) with or without α-syn deficient brain or liver cytosol before separating membrane and supernatant by centrifugation at 14,000 × g for 10 min. The supernatant was centrifuged at 100,000 × g to remove trace contaminating elements such as broken membranes or dissociated synaptic vesicles before quantifying soluble α-syn by Western blotting.
Cytosol-regulated α-Synuclein Dissociation

A. Cytosol dependent α-syn dissociation

<table>
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<tr>
<th>Cytosol (mg/ml)</th>
<th>α-syn</th>
<th>Synaptophysin</th>
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<tr>
<td>0</td>
<td></td>
<td></td>
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<tr>
<td>0.015</td>
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<tr>
<td>0.5</td>
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<tr>
<td>1.5</td>
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B. Cytosol concentration effect

C. Murine α-syn

FIGURE 2. A, cytosol-dependent α-syn dissociation from synaptosome membranes. α-Syn Tg membranes and brain α-syn-deficient cytosol were co-incubated to assess the role of cytosolic proteins on α-syn dissociation. Incubation for 10 min at 37 °C of hypotonia-lysed synaptosome membranes (100 mg of wet synaptosomal membrane weight) with various cytosol concentrations (0–1.5 mg/ml, total protein concentration) increased soluble human α-syn, as detected by human-specific antibody 211. The integral membrane protein synaptophysin was not released into the supernatant but remained associated with the membrane pellet (Membr) solubilized with 1% Triton X-100 buffer. B, quantification of cytosol concentration on dissociation of human α-syn. Mean α-syn intensity and S.E. of four independent experiments and normalized to α-syn solubilized by 1.5 mg/ml cytosol. α-syn dissociation in the presence of 1.5 mg/ml cytosol was blocked by pretreatment of membranes with LWS1 α-syn antibody (Student’s t test, p < 0.0001, n = 4), compared with 1.5 mg/ml cytosol without antibody condition, but not anti-synaptophysin antibody (Student’s t test, p > 0.3, n = 4). C, regulated dissociation of murine α-syn. Western blot probed with Syn-1 antibody showing endogenous murine α-syn from non-Tg mice also exhibited cytosol-dependent dissociation from synaptosomes membranes upon incubation for 10 min at 37 °C with 1.5 mg/ml α-syn + cytosol.

RESULTS

Cytosolic Proteins Increase α-Syn Dissociation—To identify regulators of α-syn solubility, we assessed the effects of cytosolic proteins on the interaction of α-syn with membranes. We measured α-syn dissociation from synaptosomal membranes prepared from brains of transgenic mice expressing human α-syn as the donor of membrane-bound α-syn in the absence or presence of brain cytosol derived from α-syn-deficient mice as the acceptor soluble fraction (Fig. 1A). Intact synaptosomes from α-syn transgenic mouse brain suspended in hypotonic buffer release ~30% of their total α-syn content, which comprises the soluble cytoplasmic pool (Fig. 1B). The remaining α-syn is stably associated with the membrane fraction following resuspension in isotonic buffer and is entirely susceptible to membrane disruption by detergents. The release of membrane-derived α-syn into the supernatant was markedly increased by the addition of cytosol, whereas integral membrane proteins, such as synaptophysin, were retained by the membrane pellet (Fig. 2A). Preincubation of membranes with antiserum raised to α-syn inhibited α-syn dissociation, in contrast to anti-synaptophysin antibodies or preimmune serum, neither of which had any effect (Fig. 2B). The ability to dissociate from presynaptic membranes was not exclusive to human α-syn, because endogenous α-syn from non-transgenic mice also exhibited similar cytosol-dependent dissociation from presynaptic membranes (Fig. 2C).

Comparable levels of a nonspecific protein, bovine serum albumin (BSA), did not affect α-syn solubility (Fig. 3A). However, the cytosolic activity was heat- and protease-labile as cytosol preincubated at 95 °C or pre-digested with trypsin failed to induce α-syn dissociation, suggesting that this process
Cytosolic Proteins Are Rate-limiting for α-Syn Translocation—The kinetics of α-syn dissociation from membranes were increased by raising the cytosol concentration. The appearance of soluble α-syn reached a plateau within 5–10 min, suggesting that cytosolic factors are rate-limiting under the conditions of our assay. Because the liberated α-syn represents less than 5% of the total membrane-associated α-syn, the majority of the α-syn is unaffected. Therefore, we examined whether any of the remaining bound α-syn was susceptible to cytosol-mediated dissociation by subsequent exposure to cytosol. Fig. 4B shows that additional membrane-bound α-syn could indeed be solubilized, although the amount in successive incubations declined somewhat, consistent with a reduction in the pool of extractable α-syn. If cytosol was omitted during the second incubation, no additional α-syn release was detected arguing that each dissociation event requires cytosolic factors and that α-syn is not primed for dissociation by the prior exposure to cytosol.

We tested whether cytosol used during one incubation retained activity to extract additional α-syn from fresh synaptic membranes. To distinguish between α-syn extracted during the first and second incubation, we used synaptosomes from non-Tg mice in the first incubation and measured the dissociation of endogenous murine α-syn using the syn-1 antibody (Fig. 4C, lane 1). The “used” cytosol, now containing murine α-syn, was incubated with permeabilized synaptosomes from human α-syn Tg mice that are murine α-syn-deficient. The anti-α-syn antibody 211, which is specific for the human isoform, did not reveal any human α-syn released from membranes in the presence of used cytosol (Fig. 4C, lane 2) but did so when using fresh cytosol (Fig. 4C, lane 3). These results suggest that the availability of cytosolic proteins determines the extent of α-syn dissociation.
Cytosol-regulated α-Synuclein Dissociation

α-Syn Dissociation Is Not Regulated by Ca²⁺ or by Nucleotide Triphosphates—Because many nerve terminal functions are subject to regulation by Ca²⁺ influx and the availability of metabolic energy, we examined whether α-syn translocation is affected by changes in these parameters. No significant differences on α-syn dissociation were observed in the presence or absence (with EGTA) of 1 mm Ca²⁺ and in the presence of increasing concentrations of cytosol, suggesting a Ca²⁺-independent mechanism (Fig. 5A). Next, we assessed the effects of ATP and GTP, their non-hydrolyzable analogs, and their various metabolic products in our assay. We did not observe any significant changes to α-syn dissociation in the presence of any of these compounds (Fig. 5B), suggesting that α-syn dissociation occurs independently of high energy phosphates.

DISCUSSION

Current models of α-syn lipid interaction predict that the amino-terminal portion of α-syn assembles into an amphipathic α-helix that embeds partially into membrane bilayers (8, 10, 11, 16, 34). In accord with this concept, our results show that the majority of synaptosomal α-syn is associated with membranes in vivo, although substantial portion is freely diffusible within the cytoplasm. How these bound and diffusible pools are maintained and whether regulated exchange occurs between these α-syn compartments is unclear. Here, we present evidence that α-syn stably associated with the membrane fraction can be recruited into the soluble fraction in the presence of brain cytosol. This was not due to a detergent action of cytosol because integral membrane proteins, e.g. synaptophysin, remained with the membrane pellet and the process was blocked specifically by antibodies to α-syn. Because the antisera alone had no effect on α-syn distribution in the absence of cytosol, the antibodies likely prevent access of cytosolic factors to membrane α-syn.

Moreover, predigestion of brain cytosol with trypsin or preheating at 95 °C eliminated the activity, directly implicating a
Cytosol-regulated α-Synuclein Dissociation

**FIGURE 5.** Calcium and phosphate high energy did not affect α-syn dissociation. **A**, histogram shows mean and S.E. of α-syn dissociated from synaptic membranes in the presence of different concentrations of brain cytosol supplemented with 1 mM Ca\(^{2+}\) or 1 mM EGTA. No significant difference was observed between EGTA and Ca\(^{2+}\) treatments (one-way ANOVA, p > 0.5, n = 5). **B**, the effects of 1 mM adenosine, ATP, ADP, GTP, ATP\(\gamma\)S, and GTP\(\gamma\)S, or 3 mM of AMP-CP (non-hydrolysable ADP analog) and AMP-PNP (the non-hydrolysable ATP analog) on α-syn dissociation were measured in the presence or absence of cytosol following incubation at 37 °C for 10 min. Neither nucleotide triphosphate nor their non-hydrolyzable analogs or metabolic products affected significantly basal or cytosol-induced α-syn dissociation (one-way ANOVA, with cytosol p > 0.1, n = 5; without cytosol p > 0.2, n = 5). CTRL, control.

**FIGURE 6.** Increased dissociation of A30P and A53T α-syn compared with Wt α-syn. **A**, two familial PD-linked mutations increase α-syn dissociation. Dissociation of α-syn from equivalent amounts of synaptic membranes (100 mg of wet synaptosomal membrane weight) derived from Wt, A53T, or A30P transgenic α-syn mice was measured in the presence of various concentrations of cytosol (0.15, 0.5, and 1.5 mg/ml). The top panel shows a representative Western blot of α-syn in the supernatant (S) and in the membrane pellet (P) after a 10-min incubation at 37 °C (using human specific anti-α-syn antibody 211). Synaptophysin immunoreactivity reflects synaptic membrane input. The bottom panel illustrates the combined results of four independent experiments. Dissociation of α-syn with either PD-linked mutation was significantly higher in the presence of 1.5 mg/ml cytosol (**, one-way ANOVA, Bonferroni’s test, p = 0.0012) but not in the presence of lower cytosol concentrations (0.15 and 0.5 mg/ml cytosol; one-way ANOVA, p > 0.7).

The proteins triggering α-syn dissociation are in limiting quantity in cytosol and are not regenerated under the conditions of our assay. A single exposure to synaptosome membranes was sufficient to deplete cytosolic capacity to extract membrane α-syn so that subsequent incubations with fresh membranes yielded no additional soluble α-syn. In contrast, presynaptic membranes retained ample extractable α-syn, which could be dissociated with subsequent applications of cytosol. While this suggests that the rate and extent of α-syn dissociation is primarily dependent on the availability of cytosolic factors, additional studies will be needed to determine whether some of the α-syn that remains membrane-bound is biochemically distinct from the cytosol-extracted α-syn.

The cytosolic proteins in our experiments are substantially less concentrated than presynaptic cytosol in vivo (estimated at 200 – 300 mg/ml) as a result of dilution with extraction buffers during isolation and contamination from non-neuronal cells in brain. Arguably, the portion of α-syn that can potentially be mobilized from membranes is likely to be correspondingly greater in intact cells than the 5% shifts we detected. This is compatible with a previous report by Fortin et al. (35) showing that GFP-tagged α-syn expressed in primary hippocampal neurons accumulates at synaptic boutons and that the fluorescence at photobleached boutons recovers rapidly as if α-syn has substantial steady state mobility and can be replenished from adjacent boutons. Neuronal stimulation in the presence of extracellular Ca\(^{2+}\) dispersed the α-syn fluorescence from presynapses in a tetanus toxin-sensitive manner, suggesting that α-syn translocation occurred after exocytosis and that Ca\(^{2+}\) entry per se is an insufficient trigger. We also did not observe any Ca\(^{2+}\) dependence or a direct requirement for nucleotide triphosphate cleavage in inducing α-syn dissociation. Although it is likely that post-exocytotic regulatory aspects are not recapitulated in our assay, our observations demonstrating cytosol-mediated α-syn dissociation may underlie the movement of GFP-α-syn during resting conditions or associated with exocytosis (35). Our results suggest a dynamic model of α-syn interaction.

4 W. Balch, personal communication.
Cytosol-regulated α-Synuclein Dissociation

A. Cytosolic and total α-syn levels

![Graph showing α-syn and Synaptophysin levels in WT, A30P, and A53T conditions]

B. Bound α-syn levels unaffected by PD mutations

![Graph showing α-syn and Synaptophysin levels in WT, A30P, and A53T conditions]

FIGURE 7. A, proportion of cytoplasmic and total synucleins is not affected by PD mutations. Top panel, α-Syn expression levels and distribution in cytoplasm were compared between synaptosomes derived from Wt (lanes 1–3), A30P (lanes 4–6), and A53T (lanes 7–9) α-syn Tg mice. A representative Western blot showing α-syn, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and synaptophysin in the supernatant following resuspension of 100 mg (wet tissue weight) intact synaptosomes into isosotic buffer (lanes 1, 4, and 7), hypertonic buffer (lanes 2, 5, and 8), or 1% Triton X-100 (lanes 3, 6, and 9). Bottom panel, quantification of five independent experiments measuring the cytosolic (C; from lanes 2, 5, and 8) and total (T; from lanes 3, 6, and 9) α-syn. No significant differences were observed between Wt and mutant α-syn expression (one-way ANOVA, cytosolic fraction, p = 0.51, n = 5; membrane fraction, p = 0.26, n = 5). B, left panel, α-syn immunoreactivity was compared between pellet fractions of the hypotonically-lysed synaptosomes from A (lanes 2, 5, and 8). Right panel, no significant differences were observed in the amount of membrane bound α-syn in hypotonically-lysed synaptosomes from α-syn Wt and mutant Tg mice (one-way ANOVA, p > 0.8).

with membranes, in which cytosolic proteins control the exchange of membrane-stabilized α-syn with a diffusible form, and may explain some discrepancies in previous studies regarding the proportions of soluble and bound α-syn, which ranged from equal distribution (36, 37) to virtually all soluble (13). The fact that most of the α-syn in synaptosomes is membrane-bound during steady state conditions suggests that net cytosol-induced dissociation is impeded until activation by physiological stimuli and that the equilibrium between membrane and soluble pools is balanced by regulated re-association to membranes.

The cytosolic activity that mediates α-syn dissociation clearly distinguishes between the Wt α-syn and the PD-associated mutants. Both A30P and A53T mutations doubled the cytosol-induced α-syn off-rate but had no effect on the cytosol-independent dissociation. This was unexpected because several studies, although not all (16), have previously suggested that the A30P mutation (but not A53T) impairs lipid binding (12, 14, 21, 38). Those studies measured the ability of purified recombinant α-syn to insert spontaneously into purified or artificial lipids and did not account for naturally existing cofactors or chaperones. Indeed, we did not observe differences in Wt or mutant α-syn expression in our mice or their partitioning between soluble and membrane compartments, in agreement with studies on intact cultured neurons showing no effect on membrane interaction by the A30P mutation (17) and with other transgenic lines where neither the ratio of soluble and particulate α-syn (39) nor its axonal transport (40) is affected by the A30P mutation. Furthermore, in our experiments, which employ biological membranes and include potential endogenous factors that might participate in α-syn membrane interactions, A53T α-syn was as likely to dissociate in the presence of cytosol as A30P α-syn, suggesting that the mutations may confer a common pathogenic effect that is not evident when purified components are used to assess α-syn membrane binding.

Our assay relies on α-syn-deficient cytosol as the acceptor fraction in order to detect the subtle movement that would otherwise be masked by the 30% of presynaptic α-syn that is freely diffusible in cytoplasm. Although we did not address whether other peripherally associating presynaptic proteins are also recruited into the cytosolic buffer along with α-syn, this is a likely scenario since several presynaptic proteins are transiently associated with the membrane fraction and each has a distinct mechanism that regulates its direct or indirect membrane interaction (22–24). These are mediated by either a membrane protein complex (e.g. α/β-SNAP), phosphorylation (e.g. synapsin), Ca2+ (e.g. CAPS), and nucleotide triphosphate hydrolysis (e.g. NSF, dynamin). In particular, there are similarities between the dissociation of α-syn and the behavior of rab3, a GTP-binding protein that undergoes regulated dissociation and association from vesicles with the aid of GDP-dissociation inhibitor (25–27). Rab3 lacks a transmembrane domain and its membrane-attachment is afforded by hydrophobic prenyl groups at its carboxyl terminus that insert into lipid bilayers (41). GDP-dissociation inhibitor likely shelters the prenyl moieties from aqueous cytoplasm dur-
ing extraction of rab3a from membranes. In the case of α-syn, its amino-terminal is predicted to organize into an amphipathic α-helix that permits partial insertion of hydrophobic residues into the lipid bilayer (11). Regulated dissociation from membranes would require either unfolding of the α-helix or segregation of those hydrophobic residues. Both mechanisms are compatible with a requirement for a cognate cytosolic factor that regulates α-syn solubility. Notably, the differential effect imparted by both PD-linked α-syn mutations relative to Wt α-syn in the presence of cytosol implicates the as yet undefined cytosolic activity in the pathogenic process. Our approach described here to assess α-syn membrane interactions can be used to identify and characterize the cytosolic activity that modulates α-syn sequestration into and exchange between subcellular compartments. Importantly, the underlying mechanism may be amenable to experimental manipulation as a means of influencing α-syn solubility in vivo so as to prevent its pathogenic accumulation.

REFERENCES