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Cell Biology and Pathophysiology of α-Synuclein

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α-Synuclein is an abundant neuronal protein that is highly enriched in presynaptic nerve terminals. Genetics and neuropathology studies link α-synuclein to Parkinson's disease (PD) and other neurodegenerative disorders. Accumulation of misfolded oligomers and larger aggregates of α-synuclein defines multiple neurodegenerative diseases called synucleinopathies, but the mechanisms by which α-synuclein acts in neurodegeneration are unknown. Moreover, the normal cellular function of α-synuclein remains debated. In this perspective, we review the structural characteristics of α-synuclein, its developmental expression pattern, its cellular and subcellular localization, and its function in neurons. We also discuss recent progress on secretion of α-synuclein, which may contribute to its interneuronal spread in a prion-like fashion, and describe the neurotoxic effects of α-synuclein that are thought to be responsible for its role in neurodegeneration.

α-Synuclein was identified in the electric organ of *Torpedo californica* using an antibody to purified cholinergic vesicles (Fig. 1) (Maroteaux et al. 1988). In addition to describing a presynaptic localization, Maroteaux et al. (1988) also detected α-synuclein on the nuclear envelope, hence the name synuclein from synaptic vesicles ("syn") and the nuclear envelope ("nuclein"). Note, however, that the nuclear localization has not been observed in most subsequent studies and was likely caused by an antibody contaminant. A fragment of α-synuclein comprising residues 61–95 was subsequently identified in senile plaques in Alzheimer's disease brains and was termed the non-Aβ-amyloid component (NAC) (Ueda et al. 1993). In parallel, α-synuclein mRNA was found to change specifically during song acquisition in zebra finches and was named "synelfin" because its identity with α-synuclein was not realized (George et al. 1995). β-Synuclein, the second member of the synuclein family, was identified in rat and bovine brain, where it was also localized to presynaptic nerve terminals (Nakajo et al. 1990; Tobe et al. 1992). In initial studies, β-synuclein was also named phosphoneuroprotein 14 (Jakes et al. 1994; Nakajo et al. 1993). The third member of the synuclein family, γ-synuclein, was originally identified as BCSGC1 in metastatic breast cancer (Ji et al. 1997) and

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Physiological function

Discovery of α-synuclein from Torpedo electric organ (Maroteaux et al. 1988)

Synucleins bind synaptic vesicles (Maroteaux et al. 1988; Iwai et al. 1995)

Discovery of β-synuclein (Nakajo et al. 1990; Tobe et al. 1992)

Changes in α-synuclein mRNA levels during song acquisition in zebra finches (George et al. 1995)

Synucleins are intrinsically unstructured (Weinreb et al. 1996)

Discovery of γ-synuclein (Ji et al. 1997; Buchman et al. 1998a; Ninkina et al. 1998; Lavedan et al. 1998)

α-Synuclein rescues neurodegeneration caused by CSP knockout (Chandra et al. 2005)

Synucleins are essential for long-term functioning of neurons (Anwar et al. 2011; Burré et al. 2010; Greten-Harrison et al. 2010)

α-Synuclein chaperones SNARE-complex assembly via binding to synaptobrevin-2 (Burré et al. 2010)

α-Synuclein modulates vesicle pools (Scott and Roy 2012; Diao et al. 2013; Wang et al. 2014; Burré et al. 2014)

1990

2000

2010

Pathological activity

Description of NAC in Alzheimer's disease (Ueda et al. 1993)

α-Synuclein was found as main component in Lewy bodies (Spillantini et al. 1997)

Description of the first disease-linked mutation A53T (Polymeropoulos et al. 1997)

A30P mutation (Kruger et al. 1998)

Mutations accelerate fibril formation (Conway et al. 1998; El-Agnaf et al. 1998; Narhi et al. 1999)

Detection of α-synuclein in CSF and blood plasma (Borghi et al. 2000; El-Agnaf et al. 2003)

Gene triplication (Singleton et al. 2003)

Gene duplication (Ibanez et al. 2004)

E46K mutation (Zarranz et al. 2004)

Promoter polymorphisms (Maraganore et al. 2006)

Oligomeric α-synuclein found to be elevated in plasma and CSF of PD patients (El-Agnaf et al. 2006)

Suspicion of prion-like molecular spread of α-synuclein after tissue grafting (Kordower et al. 2008; Li et al. 2008)

In vivo and in vitro models of mutant and wild-type α-synuclein spread (Desplats et al. 2009; Volpicelli-Daley et al. 2011; Luk et al. 2012; Masuda-Suzukake et al. 2013)

G51D mutation (Kiely et al. 2013)

H50Q mutation (Proukakis et al. 2013)

A53E mutation (Pasanen et al. 2014)

Figure 1. Historical timeline of α-synuclein-related findings. Marked are the major cell biological discoveries (*top*, blue) and pathobiological findings (*bottom*, red).

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was subsequently cloned both as persyn (Buchman et al. 1998a; Ninkina et al. 1998) and γ-synuclein (Lavedan et al. 1998a).

α-Synuclein became the focus of intense investigation when its central role in neurodegenerative diseases was realized (Polymeropoulos et al. 1997). α-Synuclein is now known to be involved in Parkinson's disease (PD), dementia with Lewy bodies (which contain α-synuclein aggregates as a major component), multiple system atrophy, neurodegeneration with brain iron accumulation type I, diffuse Lewy body disease, and Lewy body variant of Alzheimer's disease (Spillantini et al. 1997; Wakabayashi et al. 1997; Arawaka et al. 1998; Gai et al. 1998; see also Woerman et al. 2017). Lewy bodies containing α-synuclein are a neuropathological hallmark of PD, and missense mutations in α-synuclein (A30P, E46K, H50Q, G51D, A53E, A53T [Polymeropoulos et al. 1997; Kruger et al. 1998; Zarranz et al. 2004; Kiely et al. 2013; Proukakis et al. 2013; Pasanen et al. 2014]), as well as α-synuclein gene duplications and triplications (Singleton et al. 2003; Ibanez et al. 2004; Ferese et al. 2015), appear to cause PD. Moreover, polymorphisms in regulatory elements of the α-synuclein gene predispose individuals to PD and are linked to an early onset of the disease (Maraganore et al. 2006). Yet, despite decades of intense studies, the cell biology of α-synuclein remains largely unclear, and its specific neurodegenerative effect is poorly understood.

α-SYNUCLEIN STRUCTURE

α-, β-, and γ-synucleins are small soluble proteins (140, 134, and 127 amino acids, respectively) that bind to phospholipid membranes (Clayton and George 1998). The three synucleins contain a characteristic 11-residue sequence (consensus XKTKEGVXXXX), which is repeated seven times in α- and γ-synuclein, and six times in β-synuclein. This 11-residue repeat forms an amphipathic α-helix similar to apolipoproteins upon lipid binding (George et al. 1995). The NAC region is found within this repeated sequence. The NAC region of α-synuclein is relatively hydrophobic and aggregation-prone in human α-synuclein but not in mouse α-synuclein nor in the corresponding homologous region of human β-synuclein (Ueda et al. 1993). Yet, β-synuclein is more homologous to α-synuclein in the N-terminal sequences (74%) than γ-synuclein (67%). The acidic and glutamate-rich C-terminal sequence of synucleins is unstructured (Bertini et al. 2007; Wu et al. 2008) and was implicated in multiple protein interactions (Jensen et al. 1999; Giasson et al. 2003; Cherny et al. 2004; Fernandez et al. 2004): in ion, polycation, and polyamine binding (Paik et al. 1999; Nielsen et al. 2001; Hoyer et al. 2004; Brown 2007); in modulation of membrane binding of synucleins (Davidson et al. 1998; Jo et al. 2000; Perrin et al. 2000; Eliezer et al. 2001; Volles et al. 2001; Cole et al. 2002; Bussell and Eliezer 2003; Chandra et al. 2003; Fortin et al. 2004; Nuscher et al. 2004; Bussell et al. 2005); and in protection of α-synuclein from aggregation (Crowther et al. 1998; Park et al. 2002, 2004; see also below). It is the substrate to multiple posttranslational modifications, some of which appear to be selectively enriched in α-synuclein present in Lewy bodies (see discussion below).

All α-synuclein missense mutations are localized within the membrane-binding domain of α-synuclein containing the 11-residue repeats, although only three mutations affect lipid binding (Jo et al. 2002; Fares et al. 2014; Ghosh et al. 2014). Remarkably, the A53T substitution in human α-synuclein that causes early-onset PD (Polymeropoulos et al. 1997) is normally present in mouse α-synuclein, which has no tendency to aggregate, suggesting that the various PD-linked α-synuclein point mutations produce a neurotoxic effect that is specific to the human α-synuclein sequence context.

α-SYNUCLEIN CELLULAR POOLS

α-Synuclein exists in an equilibrium between a soluble and a membrane-bound state, with its secondary structure depending on its state (Fig. 2).

Soluble cytosolic α-synuclein is intrinsically unstructured and behaves like a natively unfolded protein (Weinreb et al. 1996; Kim 1997; Chandra et al. 2003; Fauvet et al. 2012b; Burré et al. 2013). Recently, a soluble stable tetrameric

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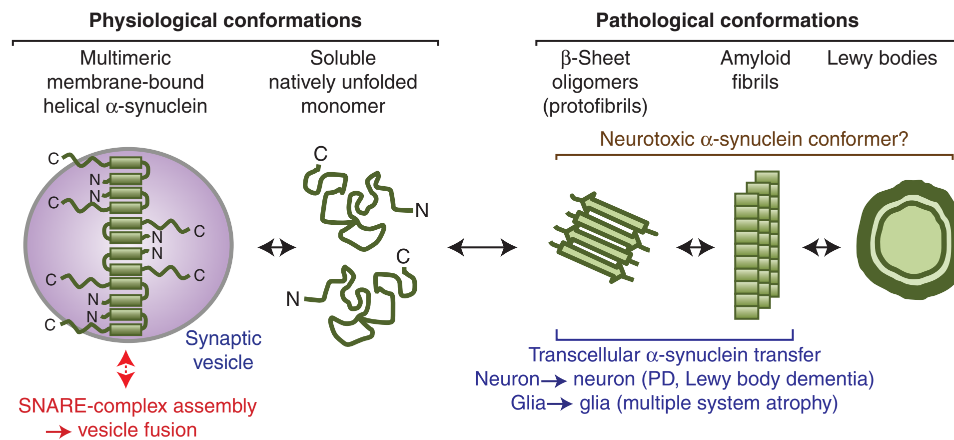

Figure 2. Schematic of α-synuclein conformations associated with its physiological function and pathological activities. Soluble α-synuclein is natively unstructured and monomeric. After binding to highly curved membranes, such as synaptic vesicles, α-synuclein undergoes a conformational change and folds into an amphipathic α-helix, which is associated with multimerization and mediates its SNARE-complex chaperoning function. Under pathological conditions, soluble α-synuclein forms β-sheet-like oligomers (protofibrils), which convert into amyloid fibrils and eventually deposit into Lewy bodies. Protofibrils and fibrils may propagate from neuron to neuron in Parkinson's disease and Lewy body dementia and from glia to glia in multiple system atrophy. (From Burré et al. 2015; reprinted, with permission, from the authors.)

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form of α-synuclein has been described in human erythrocytes as defined by analytical centrifugation but was not clearly detectable by electron microscopy (Bartels et al. 2011; Wang et al. 2011) and was not found in other preparations (Binolfi et al. 2012; Fauvet et al. 2012b; Burré et al. 2013) or by using in vivo nuclear magnetic resonance (NMR) (Fig. 3) (Theillet et al. 2016).

α-Synuclein binds to lipid membranes, such as artificial liposomes, lipid droplets, and lipid rafts. Upon lipid binding, the seven 11-residue repeat sequences of α-synuclein adopt an α-helical structure (Davidson et al. 1998; Jo et al. 2000; Perrin et al. 2000; Eliezer et al. 2001; Volles et al. 2001; Cole et al. 2002; Bussell and Eliezer 2003; Chandra et al. 2003; Fortin et al. 2004; Nuscher et al. 2004; Bussell et al. 2005). Membrane binding is likely a cooperative effect of the 11-mer sequences, as truncation of the N-terminal domain reduces lipid binding drastically (Fig. 4). α-Synuclein binding to lipid membranes requires acidic lipid head groups (Jo et al. 2000; Perrin et al. 2000; Middleton and Rhoades 2010), such as phosphatidylserine or phosphatidylinositol, suggesting an interaction of the membrane with lysines found on opposite sides of the α-synuclein α-helix. During lipid binding, α-synuclein has been reported to adopt both a single elongated α-helix and a broken α-helix depending on the membrane curvature (Bussell and Eliezer 2003; Chandra et al. 2003; Bussell et al. 2005). Specifically, membranes with a larger diameter (∼100 nm) and lower curvature induce an elongated α-helix in α-synuclein (Bussell and Eliezer 2003; Jao et al. 2004, 2008; Georgieva et al. 2008; Trexler and Rhoades 2009). In contrast, in the presence of small highly curved vesicles, α-synuclein adopts a broken α-helix conformation (Chandra et al. 2003; Ulmer et al. 2005; Borbat et al. 2006; Drescher et al. 2008; Trexler and Rhoades 2009), likely to adapt to the smaller liposome area. α-Synuclein preferentially binds to vesicles of smaller diameter (Davidson et al. 1998) and therefore associates with ∼40-nm synaptic vesicles in the brain (Maroteaux et al. 1988; Iwai et al. 1995; Kahle et al. 2000). Recently, α-synuclein was shown to oligomerize into multimers upon binding to membranes (Burré et al. 2014; Wang et al. 2014; see below).

In contrast to its physiological conformations outlined above, α-synuclein adopts a β-

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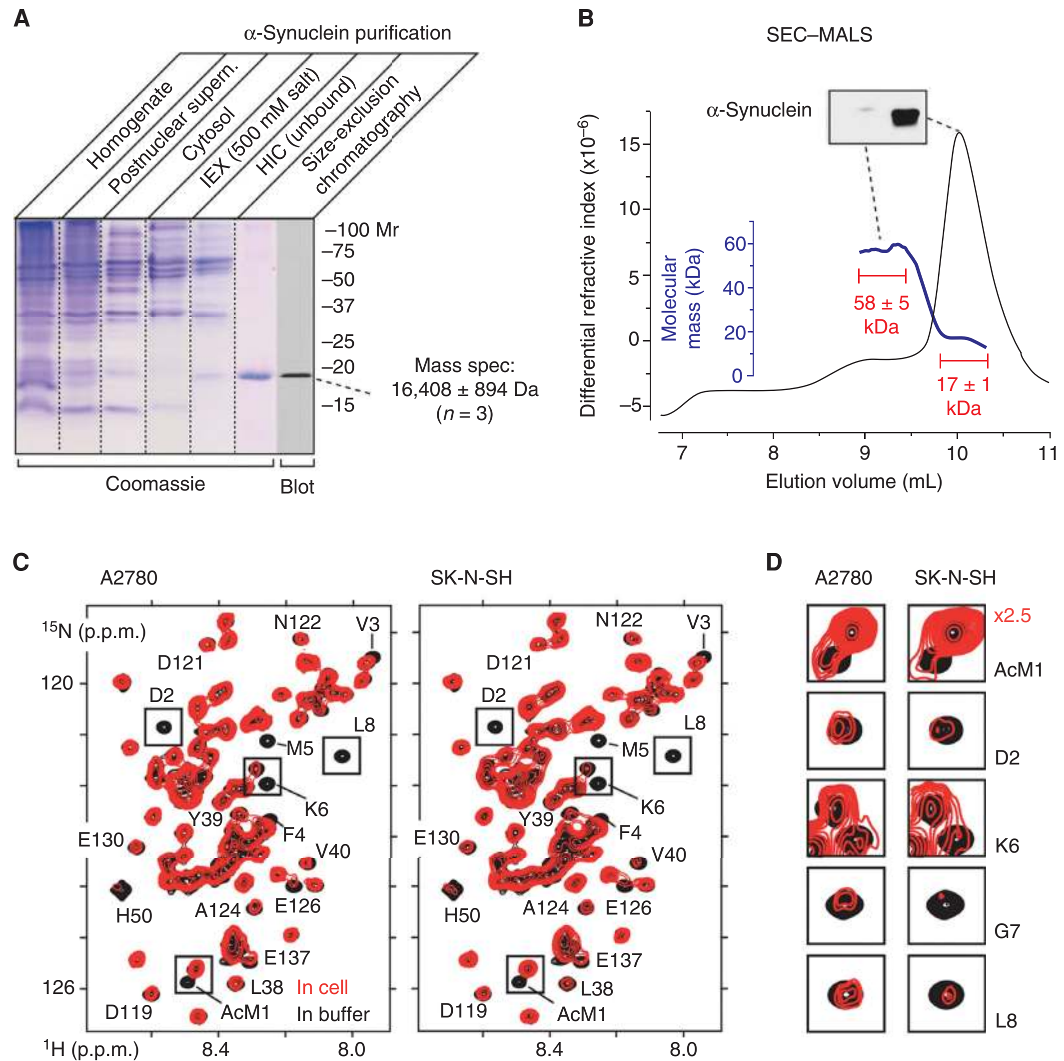

Figure 3. Soluble native α-synuclein is predominantly an unstructured monomer. (*A*) Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis of five stages of α-synuclein purification from mouse brain. Purified α-synuclein was analyzed by immunoblotting and mass spectrometry as shown. (*B*) Size-exclusion chromatography multi-angle light scattering (SEC-MALS) shows that purified α-synuclein from mouse brain tissue is largely monomeric (main peak with a mass of 17 ± 1 kDa), but includes a minor component (plateau along the left shoulder with a mass of 58 ± 5 kDa) that contains little detectable α-synuclein (see immunoblot in boxed region). Calculated masses were extracted from marked areas. (From Burré et al. 2013; reprinted, with permission, from the authors.) (*C*) Two-dimensional $^{1}H-^{15}N$ nuclear magnetic resonance (NMR) spectra of α-synuclein in A2780 and SK-N-SH cells (red, selected regions) and of isolated N-terminally acetylated α-synuclein in buffer (black). (*D*) N-terminal α-synuclein residues experiencing site-selective signal attenuations (boxed) are expanded, with in-cell NMR contours plotted at 2.5-fold lower levels (red). In-cell NMR cross-peaks are superimposed with reference NMR signals of N-terminally acetylated α-synuclein in buffer (black), confirming the presence of this modification in mammalian cells. IEX, Anion exchange chromatography; HIC, hydrophobic interaction chromatography; AcM1, acetylated Met1. (From Theillet et al. 2016; reprinted, with permission, from Nature Publishing Group © 2016.)

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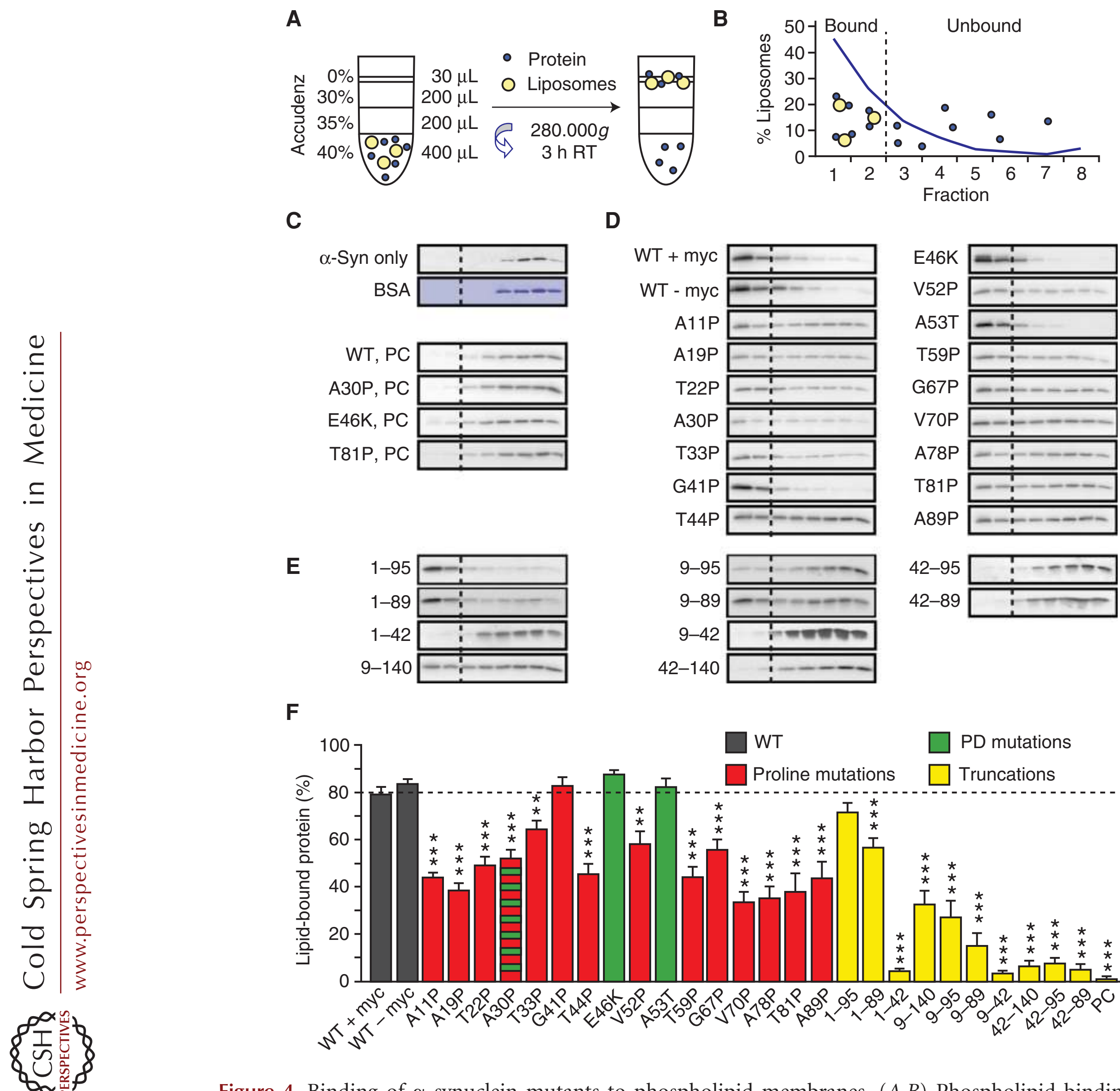

Figure 4. Binding of α-synuclein mutants to phospholipid membranes. (*A*,*B*) Phospholipid binding assay. Liposomes mixed with wild-type (WT) and mutant α-synuclein were floated by density gradient centrifugation (*A*). Based on liposome distribution in the gradient (*B*), the *top* two fractions 1 and 2 were defined as lipid-bound fractions. (*C*) Lack of flotation of bovine serum albumin (BSA) and α-synuclein in the absence of liposomes or with uncharged liposomes, analyzed by Coomassie staining or by immunoblotting with antibodies to the myc-epitope fused to α-synuclein. (*D*–*F*) Quantitation of phospholipid binding by WT and mutant α-synuclein. Flotation of α-synuclein point mutants (*D*) and truncations (*E*) with liposomes was quantitated as the sum of the *top* two fractions, and was plotted as the percentage of total α-synuclein in the gradient (*F*). Data are means ± SEM ($^{**}P < 0.01$, $^{***}P < 0.001$ by Student's *t*-test; $n = 6$ independent experiments). RT, Room temperature. (From Burré et al. 2012; reprinted, with permission, from the authors.)

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sheet amyloid conformation under pathological conditions. This β-sheet conformation is associated with α-synuclein aggregation, fibril formation, and deposition into Lewy bodies (Conway et al. 1998, 2000; El-Agnaf et al. 1998; Narhi et al. 1999; Rochet et al. 2000; Ding et al. 2002; Lashuel et al. 2002; Greenbaum et al. 2005; Fredenburg et al. 2007; Uversky 2007; Yonetani et al. 2009). The β-sheet conformation is thought to be neurotoxic, but the exact nature of the neurotoxic species remains unknown (Fig. 2).

α-SYNUCLEIN POSTTRANSLATIONAL MODIFICATIONS

α-Synuclein is subject to multiple posttranslational modifications, mostly within its carboxy-terminal tail, including phosphorylation, oxidation, acetylation, ubiquitination, glycation, glycosylation, nitration, and proteolysis, resulting in changes in protein charge and structure, and leading to alterations in binding affinities with other proteins and lipids and overall protein hydrophobicity.

Phosphorylation

Phosphorylation of α-synuclein may regulate its structure, membrane binding, oligomerization, fibril formation, and neurotoxicity (Fujiwara et al. 2002; Anderson et al. 2006). α-Synuclein is constitutively phosphorylated, with serine 87 and serine 129 being major phosphorylation sites (Okochi et al. 2000; Pronin et al. 2000; Fujiwara et al. 2002; Kahle et al. 2002; Takahashi et al. 2003a; Chen and Feany 2005; Anderson et al. 2006; Kim et al. 2006; Ishii et al. 2007; Paleologou et al. 2010); repeated cycles of phosphorylation and dephosphorylation occur in vivo. Phosphorylation at S129 and S87 has been shown to inhibit aggregation of α-synuclein (Waxman and Giasson 2008; Paleologou et al. 2010). Similarly, tyrosine phosphorylation at Y125, Y133, and Y135 is associated with suppression of α-synuclein aggregation and toxicity (Ellis et al. 2001; Nakamura et al. 2001; Ahn et al. 2002; Negro et al. 2002; Takahashi et al. 2003b; Chen and Feany 2005; Chen et al. 2009). In addition, phosphorylation of α-synuclein at S129 and Y125 affects protein–protein interactions (McFarland et al. 2008). The exact kinases and phosphatases mediating α-synuclein phosphorylation and dephosphorylation remain unknown. Yet, a variety of in vitro and cell-based studies have identified several kinases capable of phosphorylating α-synuclein, including phosphorylation of α-synuclein at S87 by casein kinase I (Okochi et al. 2000) and Dyrk1A (Kim et al. 2006); at S129 by casein kinase I and II (Okochi et al. 2000), G-protein-coupled receptors 1, 2, 5, and 6 (Pronin et al. 2000), LRRK2 (Qing et al. 2009), and polo-like kinases (Inglis et al. 2009; Mbefo et al. 2010); at Y125 by Fyn (Nakamura et al. 2001), Syk (Negro et al. 2002), Lyn (Negro et al. 2002), c-Frg (Negro et al. 2002), and Src tyrosine kinases (Ellis et al. 2001); and at Y126 and Y133 by Syk tyrosine kinase (Negro et al. 2002).

Acetylation

Amino-terminal acetylation of α-synuclein is seen both in healthy and PD individuals, and increases its helical folding propensity, its affinity for membranes, and its resistance to aggregation (Kang et al. 2012; Maltsev et al. 2012; Bartels et al. 2014; Dikiy and Eliezer 2014), mediated by attachment of an acetyl group to the α-amino group of the first amino acid of α-synuclein (Fauvet et al. 2012a; Kang et al. 2012; Maltsev et al. 2012; Burré et al. 2013).

Sumoylation

α-Synuclein is primarily modified by SUMO1 via monosumoylation (Dorval and Fraser 2006). Sumoylation of α-synuclein occurs at only one site, which is yet to be identified, at the N-terminus of the protein. The functional significance remains unknown and controversial: Sumoylation has been reported both to inhibit aggregation of α-synuclein by promoting its solubility (Krumova et al. 2011; Shahpasandzadeh et al. 2014) and to promote aggregation (Kim et al. 2011; Oh et al. 2011).

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Ubiquitination

The role of ubiquitination in modulating α-synuclein aggregation remains poorly understood. α-Synuclein colocalizes with ubiquitin in Lewy bodies (Mezey et al. 1998; Gomez-Tortosa et al. 2000), Lewy neurites (Gomez-Tortosa et al. 2000), and glial inclusions in multiple system atrophy (Gai et al. 1998). Four E3- and E4-type ubiquitin ligases have been shown to ubiquitinate α-synuclein: Parkin, ubiquitin carboxy-terminal hydrolase L1 (UCH-L1), C-terminus of Hsp70 interacting protein (CHIP), and SIAH (Liu et al. 2002; Liani et al. 2004; Lee et al. 2008; Rott et al. 2008; Tetzlaff et al. 2008). Parkin and UCH-L1 are linked to PD themselves (Leroy et al. 1998; Shimura et al. 2000), and Parkin and SIAH are present in Lewy bodies (Liani et al. 2004; Bandopadhyay et al. 2005). In vitro, α-synuclein is ubiquitinated at K10, K21, K23, K32, K34, K43, and K96, with K21, K23, K32, and K34 being major sites (Nonaka et al. 2005; Rott et al. 2008). Studies in rat brain revealed major ubiquitination of K21 and K23 (Nonaka et al. 2005).

Glycation

Advanced glycation end products and α-synuclein colocalize in the brains of PD patients (Munch et al. 2000), and glycation of α-synuclein has been proposed to be a pathological hallmark of Lewy bodies (Munch et al. 2000; Shaikh and Nicholson 2008). Advanced glycation end products (Munch et al. 2000; Shaikh and Nicholson 2008), modification of α-synuclein with dicarbonyl compounds glyoxal and methylglyoxal (Lee et al. 2009), and ribosylation of α-synuclein (Chen et al. 2010) induce cross-linking of recombinant α-synuclein in vitro, accelerating aggregation, and causing cytotoxicity (Munch et al. 2000; Shaikh and Nicholson 2008; Chen et al. 2010; Padmaraju et al. 2011).

Glycosylation

α-Synuclein may be *O*-glycosylated in the brain at residues 53, 64, 72, and 87 (Wang et al. 2010; Alfaro et al. 2012). The biological and pathological roles of α-synuclein glycosylation remain unknown, although a recent report suggests that glycosylation of α-synuclein in the NAC domain inhibits aggregation (Marotta et al. 2012).

Nitration and Oxidation

Nitration and oxidation have been implicated in the pathogenesis of PD and diffuse Lewy body disease. Yet, it is unclear whether they are a primary event leading to aggregation of α-synuclein, or whether they occur upon reaction of reactive nitrogen species with preformed fibrils. α-Synuclein contains four tyrosines, Y39, Y125, Y133, and Y136, with Y125 and Y39 being major nitration targets (Takahashi et al. 2002; Danielson et al. 2009). Nitration of α-synuclein is believed to induce its oligomerization through cross-linking via oxidation of tyrosine to dityrosine, which may serve as a basis for the formation of larger α-synuclein aggregates (Giasson et al. 2000). Yet, nitration inhibits fibril formation of α-synuclein (Norris et al. 2003; Yamin et al. 2003; Hodara et al. 2004) and prevents fibrillation of nonmodified α-synuclein (Yamin et al. 2003), suggesting a protective effect (Yamin et al. 2003). Nitration has also been reported to inhibit binding of α-synuclein to lipid vesicles (Hodara et al. 2004; Sevcsik et al. 2011).

Proteolysis

C- and N-terminally truncated versions of α-synuclein are present in the brains of healthy individuals and PD patients (Baba et al. 1998; Li et al. 2005; Liu et al. 2005a; Lewis et al. 2010; Muntane et al. 2012), suggesting that α-synuclein truncation occurs under physiologically relevant conditions. Lewy bodies contain mostly full-length α-synuclein, with the addition of small amounts of a C-terminally truncated species (Baba et al. 1998; Spillantini et al. 1998; Crowther et al. 1998; Campbell et al. 2001; Li et al. 2005; Liu et al. 2005a). C-terminal truncation of α-synuclein increases its aggregation propensity (Rochet et al. 2000; Serpell et al. 2000; Murray et al. 2003; Hoyer et al. 2004; Li

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et al. 2005), because of the lack of charge balance between the C- and N-terminus (Bertoncini et al. 2005; Pawar et al. 2005), and could serve as the nucleus to seed aggregation of full-length α-synuclein. Yet, the cleaving protease remains enigmatic. Neurosin (Ogawa et al. 2000; Iwata et al. 2003; Kasai et al. 2008) and calpain (Mishizen-Eberz et al. 2003; Dufty et al. 2007) are candidates because of their colocalization in Lewy bodies. In addition, cathepsin D (Takahashi et al. 2007; Sevlever et al. 2008), matrix metalloproteases (Sung et al. 2005; Levin et al. 2009), and ubiquitin-independent degradation of α-synuclein by the proteasome (Tofaris et al. 2001) have been implicated in C-terminal proteolytic cleavage of α-synuclein.

Summary of α-Synuclein Posttranslational Modifications

The description above shows that α-synuclein can be posttranslationally modified at many of its 140 residues and by a variety of cellular enzymes. Many of the above studies have used overexpression systems and have neglected the physiologically relevant localization of α-synuclein, the synapse, when testing for the activity of kinases, phosphatases, or other enzymes. Thus, many of these modifications likely represent rare events. It is clear, however, that the structure of α-synuclein, and thus its (dys-) function, can be heavily altered by posttranslational modifications. It remains to be determined which posttranslational modifications are physiologically relevant and which arise as a function of α-synuclein-associated pathology.

α-SYNUCLEIN EXPRESSION AND LOCALIZATION

α- and β-synucleins are expressed predominantly in the brain (Jakes et al. 1994), particularly in the neocortex, hippocampus, striatum, thalamus, and cerebellum (Nakajo et al. 1994; Iwai et al. 1995). Although γ-synuclein is detected in the brain (Buchman et al. 1998b), it is primarily expressed in the peripheral nervous system—in primary sensory, sympathetic, and motor neurons (Ji et al. 1997), as well as in the olfactory epithelium (Duda et al. 1999). It can also be expressed in ovarian tumors (Lavedan et al. 1998). γ-Synuclein, however, is also fairly robustly expressed in non-neuronal tissues and is increased in expression in some cancers (Ji et al. 1997; Jia et al. 1999; Liu et al. 2005b; Ahmad et al. 2007). Moreover, α-synuclein, although highly enriched in the nervous system, is not limited to nervous tissues. α-Synuclein has been detected in muscle, kidney, liver, lung, heart, testis, blood vessels, cerebrospinal fluid (CSF), blood plasma, platelets, lymphocytes, and red blood cells (Ueda et al. 1993; Jakes et al. 1994; Hashimoto et al. 1997; Askanas et al. 2000; Shin et al. 2000; Li et al. 2002; Tamo et al. 2002; Kim et al. 2004a; Ltic et al. 2004; Nakai et al. 2007). Thus, similar to proteins such as *N*-ethylmaleimide-sensitive factor (NSF) and synaptosomal-associated proteins (SNAP) (which function in ATP-dependent dissociation of soluble NSF attachment protein receptor [SNARE] complexes), synucleins are enriched in neurons but are ubiquitously expressed.

Within the nervous system, α-synuclein is universally expressed throughout the brain (Lavedan 1998) in a developmentally regulated manner. In rodents, α-synuclein mRNA expression begins in late embryonic stages, reaches a peak in the first few postnatal weeks, and then declines gradually (Kholodilov et al. 1999; Petersen et al. 1999). α-Synuclein protein levels generally mirror mRNA levels, but remain high during adulthood (Petersen et al. 1999). α-Synuclein levels in adults can be altered by various stimuli such as the herbicide paraquat (Manning-Bog et al. 2002), developmental injury to the brain (Kholodilov et al. 1999), neurotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) (Vila et al. 2000), growth factors such as nerve growth factor and basic fibroblast growth factor (Clough and Stefanis 2007), and dopamine (Gomez-Santos et al. 2003, 2005). Overall, little is known about the characteristics and mechanisms of α-synuclein expression, a subject that is of potential translational importance, given the role α-synuclein levels play in PD predisposition.

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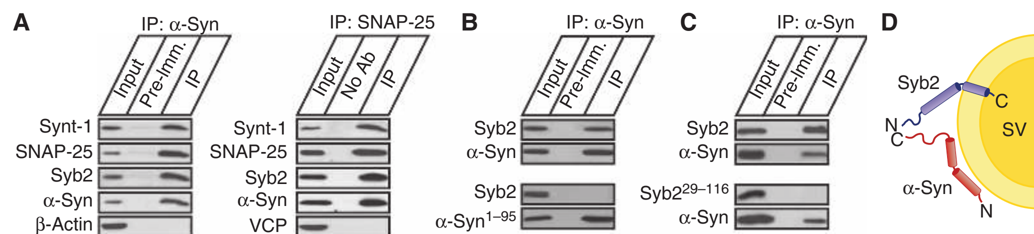

Figure 5. α-Synuclein directly binds to synaptobrevin-2/vesicle-associated membrane protein 2 (VAMP2) in SNARE complexes. (*A*) Coimmunoprecipitation of α-synuclein with SNARE complexes reconstituted in HEK293T cells. Cell lysates were immunoprecipitated with antibodies to (*left*) α-synuclein or (*right*) SNAP-25 and analyzed by means of immunoblotting. (*B*,*C*) The C terminus of α-synuclein directly binds to the N terminus of synaptobrevin-2. α-Synuclein was immunoprecipitated from HEK293T cells coexpressing full-length (α-Syn) or C-terminally truncated α-synuclein (α-Syn^{1-95}) with full-length (Syb2) or N-terminally truncated synaptobrevin-2 ($Syb2^{29-116}$). Immunoprecipitates were analyzed by means of immunoblotting of α-synuclein and synaptobrevin-2. (*D*) Diagram of the α-synuclein/synaptobrevin-2 complex on synaptic vesicles (SVs). (From Burré et al. 2010; reprinted, with permission, from the authors.)

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Localization to the Synapse

During the development of cultured neurons, α-synuclein is first localized to the soma of the immature neuron and then becomes concentrated in presynaptic terminals as synapses are being formed in murine (Withers et al. 1997; Hsu et al. 1998) and in humans (Bayer et al. 1999; Galvin et al. 2001). Interestingly, although α-synuclein is one of the best markers for presynaptic terminals, it is among the last presynaptic proteins to become enriched in terminals (Withers et al. 1997), suggesting that it does not play a central function in synapse development. Targeting of α-synuclein to the synapse may be mediated by its preference for synaptic vesicle membranes (Maroteaux et al. 1988; Jensen et al. 1999) and/or binding to the vesicular SNARE protein synaptobrevin-2 (Fig. 5) (Burré et al. 2010). In support of the latter hypothesis, knockout of synaptobrevin-2 decreases targeting of α-synuclein to the synapse (Burré et al. 2012).

Localization to Mitochondria

Multiple studies reported that α-synuclein localizes to and binds to mitochondria (Li et al. 2007; Cole et al. 2008; Devi et al. 2008; Nakamura et al. 2008; Liu et al. 2009). Because mitochondria, different from synaptic vesicles, are not specific to nerve terminals, it is difficult to reconcile the presynaptic localization of α-synuclein with a mitochondrial function. The potential function of α-synuclein in mitochondria is unclear because both overexpression and loss of α-synuclein was proposed to cause mitochondrial dysfunction (Ellis et al. 2005; Smith et al. 2005; Martin et al. 2006; Stichel et al. 2007). Overexpression of α-synuclein in multiple cell types, including neurons, can lead to fragmentation of mitochondria (Kamp et al. 2010; Nakamura et al. 2011), not by preventing fusion, but by promoting mitochondrial fission (Nakamura et al. 2011). In vitro, membranes containing the mitochondrial phospholipid cardiolipin can be fragmented by oligomerized α-synuclein (Nakamura et al. 2011). Interestingly, β- and γ-synuclein can also affect mitochondrial morphology, although to a lesser extent than α-synuclein (Nakamura et al. 2011), which suggests the involvement of the N-terminal membrane-binding domain, which is highly conserved between the three synuclein isoforms. The A53T mutant version of α-synuclein, when overexpressed in primary neurons, up-regulates autophagic engulfment of mitochondria (mitophagy) (Choubey et al. 2011), and mitochondrial degeneration was reported in transgenic mice overexpressing A53T α-synuclein (Martin et al. 2006).

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On the molecular level, α-synuclein overexpression appears to inhibit complex I (Li et al. 2007; Devi et al. 2008; Nakamura et al. 2008; Liu et al. 2009; Chinta et al. 2010; Loeb et al. 2010), which, in combination with the ability of α-synuclein to disrupt mitochondrial membrane integrity, may lead to increased production of reactive oxygen species (ROS) (Hsu et al. 2000; Junn and Mouradian 2002; Winklhofer and Haass 2010). This pathogenic cascade of α-synuclein may overlap with other mitochondrial/ROS-related neuropathology that causes PD, such as PINK1, parkin, and LRRK mutations, or sporadic PD caused by mitochondrial toxins. The importance of mitochondrial dysfunction in synucleinopathies is further suggested by studies in which α-synuclein-dependent mitochondrial dysfunction or ROS production were counteracted by molecular or pharmacological means, which led to neuroprotective effects in mammalian systems (Hsu et al. 2000; Clark et al. 2010; Liu et al. 2011) and in *Drosophila* (Wassef et al. 2007; Botella et al. 2008).

Localization to the Nucleus, Endoplasmic Reticulum, and Golgi

α-Synuclein was named in part because of a presumed localization to the nucleus (Maroteaux et al. 1988). Since then, the reports of nuclear localization of α-synuclein have not been consistent (Mori et al. 2002; Yu et al. 2007). Phosphorylation of α-synuclein at S129 was proposed to increase its nuclear localization in transgenic mice overexpressing α-synuclein with the A30P mutation (Schell et al. 2009). In the nucleus, α-synuclein is reported to inhibit histone acetylation (Kontopoulos et al. 2006), and histone deacetylase (HDAC) inhibitors were able to rescue neurotoxicity caused by α-synuclein in cell culture and in transgenic *Drosophila* (Kontopoulos et al. 2006). In agreement, inhibition or siRNA-mediated knockdown of Sirtuin-2, an HDAC, protected against α-synuclein mediated dopaminergic neuron death in culture and transgenic *Drosophila* models (Outeiro et al. 2007).

α-Synuclein was also proposed to associate with the Golgi complex and various secretory and endosomal compartments other than synaptic vesicles. Endoplasmic reticulum (ER) associated stress was observed in a cell culture model of A53T α-synuclein-induced cell death (Smith et al. 2005). Golgi fragmentation correlated with small, prefibrillar oligomers of α-synuclein (Gosavi et al. 2002). Blockade of ER–Golgi traffic was found to be involved in α-synuclein toxicity in yeast, and could be rescued by overexpression of rab1—a protein involved in ER–Golgi traffic (Cooper et al. 2006). Overexpression of α-synuclein severely delayed ER–Golgi transport in non-neuronal cells by inhibiting ER/Golgi SNARE protein function (Thayanidhi et al. 2010). These rab and SNARE effects of α-synuclein in non-neuronal cells may derive from its native function on the presynaptic membranes.

Localization to the Cytoskeleton

α-Synuclein interacts with multiple cytoskeletal components in vitro. The helical membrane-binding domain of α-synuclein, when bound to lipids, associates with cellular proteins like tubulin (Alim et al. 2002; Zhou et al. 2010), kinesin light chain, dynein heavy chain, and septin-4 (Woods et al. 2007). However, the proposed effects of α-synuclein on tubulin polymerization are unclear, with inhibition reported by some (Lee et al. 2006a; Zhou et al. 2010) and enhancement by others (Alim et al. 2002). Importantly, aggregates of the microtubule-associated protein tau have been implicated in certain cases of PD (Kotzbauer et al. 2004), and tau shows a high association with PD in genome-wide association studies (Nalls et al. 2011). α-Synuclein enhances phosphorylation of tau (Jensen et al. 1999; Haggerty et al. 2011; Qureshi and Paudel 2011), and tau and α-synuclein can seed aggregation of each other (Giasson et al. 2003), possibly accelerating the neuropathological cascade.

α-Synuclein Expression and Localization Summary

The description above shows that α-synuclein is a ubiquitously expressed protein that is highly

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enriched in neurons, in which α-synuclein is largely concentrated in presynaptic terminals where it is localized to synaptic vesicles. In addition to these observations, a significant number of additional localizations have been proposed for α-synuclein, as described above, largely based on overexpression studies. Many of these results likely represent rare events whose significance is unclear. For example, synaptic vesicles in presynaptic terminals are not in contact with microtubules, suggesting that the vast majority of α-synuclein that is on synaptic vesicles cannot be bound to microtubules or tau. Similarly, mitochondria are not nearly as enriched in presynaptic terminals as synaptic vesicles or as endogenous α-synuclein, arguing against a significant association between α-synuclein and mitochondria. The same argument applies even more to the endoplasmic reticulum, which is not at all enriched in terminals, and to the nucleus and Golgi apparatus, which are absent from terminals. Thus, the very fact that endogenous α-synuclein is normally enriched in presynaptic terminals provides good evidence against many of the localizations proposed for α-synuclein other than synaptic vesicles.

α-Synuclein and Protein Degradation Mechanisms

It is proposed that α-synuclein is degraded by multiple mechanisms, from the ubiquitin proteasome pathway and autophagy–lysosome system to extracellular metalloproteases. Initial studies showed that proteasome inhibition led to accumulation of α-synuclein (Bennett et al. 1999; McLean et al. 2001). Then the autophagy–lysosomal pathway was also implicated in α-synuclein degradation (Webb et al. 2003; Cuervo et al. 2004; Lee et al. 2004), which was further narrowed down to chaperone-mediated autophagy (CMA) (Cuervo et al. 2004; Bandyopadhyay and Cuervo 2007).

The ability of α-synuclein to block various degradation pathways, especially in its oligomeric/aggregate form, has garnered considerable research interest, which is possibly because of the attractive notion that this would create a vicious cycle leading to further accumulation of α-synuclein oligomers/aggregates. Overexpression of mutant α-synuclein in cell lines revealed inhibition of the ubiquitin–proteasome system (Stefanis et al. 2001; Tanaka et al. 2001), sensitization to proteasome inhibitors because of decreased proteasome activity (Petrucelli et al. 2002), or no effect on the proteasome (Martin-Clemente et al. 2004). Any effect on the proteasome may be direct, as interactions between monomeric and aggregated α-synuclein and the proteasomal subunit S6′ of the 19S regulatory complex as well as subunits of the 20S proteasome have been observed (Snyder et al. 2003; Lindersson et al. 2004). α-Synuclein aggregates of various sizes, from soluble oligomers to filaments, were shown to inhibit proteasome activity (Lindersson et al. 2004; Emmanouilidou et al. 2010b).

Inhibition of CMA by α-synuclein has also been widely reported, with decreased levels of CMA proteins LAMP2A and Hsc70 in PD brains (Alvarez-Erviti et al. 2010). Although wild-type (WT) α-synuclein is translocated into lysosomes for degradation via CMA, A30P and A53T α-synuclein mutants inhibit uptake (Cuervo et al. 2004), and inhibition of CMA by α-synuclein causes a compensatory increase in macroautophagy (Xilouri et al. 2009). However, inhibition of macroautophagy by WT α-synuclein has also been reported (Winslow et al. 2010).

α-SYNUCLEIN IN EXTRACELLULAR SPACE AND ITS PRION-LIKE SPREAD

Because early symptoms of PD were highly stereotyped, α-synuclein aggregates were thought to exert their pathogenic effect in a cell-autonomous and brain-area-specific manner. However, this model was first challenged by a pattern of anatomical progression of Lewy body pathology and neuron loss beginning in brainstem nuclei, extending to the midbrain, and finally to cortical areas (Braak et al. 2004), which may account for the symptomatic progression of PD beyond the most obvious initial motor symptoms, such as depression, autonomic and sensory dysfunction, and dementia. One of the

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earliest indications of extracellular α-synuclein was its detection in the extracellular fluids such as CSF and blood plasma (Borghi et al. 2000; El-Agnaf et al. 2003). The amount of extracellular α-synuclein, however, was not clearly correlated with PD, as elevation (Lee et al. 2006b), as well as reduction (Tokuda et al. 2010), of α-synuclein was reported in blood samples from PD patients. Although oligomeric α-synuclein is found at elevated levels in the plasma and CSF of PD patients (El-Agnaf et al. 2006), it remains uncertain whether α-synuclein in bodily fluids is a useful indicator/biomarker of disease.

Suspicion of prion-like molecular spread of α-synuclein came from postmortem studies of PD patients who had received fetal brain tissue grafts (Lindvall et al. 1994; Olanow et al. 2003). In patients who died 11–16 years after transplantation, the grafted neurons showed Lewy bodies that were essentially identical in content and character to those in the PD-afflicted brain (Kordower et al. 2008; Li et al. 2008; Kurowska et al. 2011). Likely, this neuron-to-neuron spread appears to be a prion-like propagation of α-synuclein, with evidence accumulating from in vitro and in vivo models of not only mutant and WT α-synuclein spread (Desplats et al. 2009; Luk et al. 2012; Masuda-Suzukake et al. 2013), but also from extraneuronal spread of Aβ (Petkova et al. 2005; Jucker and Walker 2011) and tau (Clavaguera et al. 2009; Frost et al. 2009).

Release of α-synuclein appears to be through unconventional exocytosis pathways. Although the molecular mechanisms are not well understood, a growing number of cytosolic proteins seem to be released into the extracellular space (Nickel 2003). Some evidence indicates that α-synuclein may be released with exosomes—luminal vesicles of multivesicular bodies (MVBs), which are classically understood to be on their way to degradation in lysosomes (Emmanouilidou et al. 2010a). This release appears to be calcium-dependent (Lee et al. 2005; Paillusson et al. 2013), providing a neuronal activity-dependent mechanism for α-synuclein exocytosis, and may suggest spread along synaptically connected neurons.

Once in the extracellular space, α-synuclein may be removed by proteolysis by extracellular enzymes such as matrix metalloproteases (Sung et al. 2005) or via uptake by surrounding cells. Extracellular α-synuclein can be endocytosed by neurons and microglia (Sung et al. 2001; Zhang et al. 2005), although non-endocytosis-dependent uptake has also been reported (Ahn et al. 2006a). Fibrils of recombinant α-synuclein can also be endocytosed by neurons and lead to aggregates of Lewy-body-like pathology in cells expressing endogenous levels of α-synuclein (Volpicelli-Daley et al. 2011). Exocytosis and uptake of α-synuclein may be an important mechanism for the progression and amplification of degenerative changes in synucleinopathies from a few cells to the surrounding tissue, or it may also have a biological function that is not yet known (see also Hasegawa et al. 2016; Tofaris et al. 2016).

α-SYNUCLEIN FUNCTION

In addition to its well-validated binding to negatively charged phospholipids (Davidson et al. 1998; Bussell and Eliezer 2003; Chandra et al. 2003), α-synuclein has been reported to interact with a variety of proteins and to perform a number of functions in conjunction with these protein interactions. These binding and functional activities include binding and inhibition of phospholipase D (Jenco et al. 1998; Ahn et al. 2002; Payton et al. 2004; Gorbatyuk et al. 2010); regulation of the interaction of the small GTP-binding protein rab3 with membranes, especially synaptic vesicles (Chen et al. 2013); binding to the SNARE-protein synaptobrevin-2 and chaperoning SNARE-complex assembly (Figs. 5 and 6) (Burré et al. 2010); binding to and regulation of tyrosine hydroxylase (TH) (Masliah et al. 2000; Kirik et al. 2002; Perez et al. 2002; Baptista et al. 2003; Yu et al. 2004); binding to DJ-1 (Zondler et al. 2014) and to synphilin (Engelender et al. 1999; McLean et al. 2001; Ribeiro et al. 2002); regulation of microtubules via binding to tubulin (Lee et al. 2006a); and enhancement of tau phosphorylation (Jensen et al. 1999; Haggerty et al. 2011; Qureshi and Paudel 2011). Yet, the

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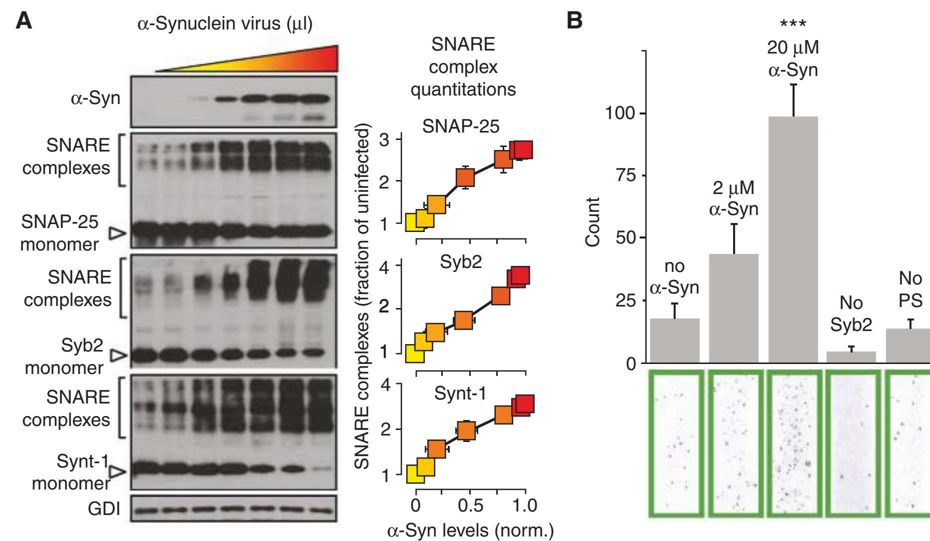

Figure 6. α-Synuclein boosts SNARE-complex assembly and clusters liposomes. (*A*) Linear relationship between α-synuclein levels and SNARE-complex assembly in α, β, γ-synuclein triple-knockout neurons infected at DIV4 with increasing amounts of lentivirus expressing α-synuclein and analyzed by means of immunoblotting of nonboiled samples at DIV17 ($n = 3$ to 6 cultures). (From Burré et al. 2010; reprinted, with permission, from the authors.) (*B*) Native α-synuclein promotes liposome vesicle clustering in a concentration-dependent manner by binding to both synaptobrevin-2 and anionic membranes. Bar graph: Quantitation of interacting vesicles. (*Bottom* panel) Representative fluorescence images of interacting vesicles on the imaging surface. Data are means ± SD ($^{***}P < 0.001$ by Student's *t*-test; $n = 15$ random imaging locations in the sample channel). (From Diao et al. 2013; reprinted, with permission, from the authors.)

physiological significance of most of these interactions remains unclear.

Lipid Transport, Lipid Packing, and Membrane Biogenesis

The binding of α-synuclein to phospholipids and the similarity of α-synuclein with class A2 apolipoproteins suggests a role in lipid transport. α-Synuclein has been reported to bind to fatty acids (Sharon et al. 2001), and may thus serve as a fatty acid transporter between the cytosol and membrane compartments, although other studies suggest the contrary (Lucke et al. 2006). Furthermore, α-synuclein has been shown to induce membrane curvature and convert large vesicles into highly curved membrane tubules and vesicles, as would be expected for a lipid-binding protein (Varkey et al. 2010; Westphal and Chandra 2013). In addition, α-synuclein has been reported to be a specific inhibitor of phospholipases D1 and D2 in vitro and in vivo (Jenco et al. 1998; Ahn et al. 2002; Payton et al. 2004; Gorbatyuk et al. 2010), suggesting that α-synuclein may be involved in cleavage of membrane lipids and membrane biogenesis. Moreover, it has been suggested that α-synuclein senses lipid-packing defects and affects lipid packing (Kamp and Beyer 2006; Ouberai et al. 2013), indicating that besides binding to membranes, α-synuclein may be able to actively remodel them.

Molecular Chaperone Activity

The biochemical structure of α-synuclein predicts a function as a molecular chaperone capable of binding to other intracellular proteins. This hypothesis was strengthened by three observations: First, α-synuclein shares structural and functional homology with the 14-3-3 family of molecular chaperone proteins (Ostrerova et al. 1999). Second, via its C-terminal domain, α-synuclein suppresses the aggregation of ther-

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mally denatured proteins (Kim et al. 2000, 2002, 2004b; Souza et al. 2000; Rekas et al. 2004; Ahn et al. 2006b), and overexpression of α-synuclein protects dopaminergic neurons from oxidative stress and apoptosis (da Costa et al. 2000; Kanda et al. 2000). Third, α-synuclein rescues the lethal neurodegeneration caused by knockout of the cochaperone CSPα in mice by chaperoning assembly of synaptic SNARE complexes (Chandra et al. 2005; Burré et al. 2010). This function of α-synuclein is essential for long-term functioning of neurons, as α-, β-, γ-synuclein triple-knockout mice have reduced SNARE-complex assembly, show neuropathological signs, and have shortened survival (Burré et al. 2010; Greten-Harrison et al. 2010; Anwar et al. 2011).

Vesicle Trafficking

A large body of evidence from studies in worm, yeast, fly, and mouse models shows that α-synuclein is associated with defects in vesicle trafficking. In yeast, α-synuclein inhibits ER–Golgi trafficking, which can be rescued by overexpression of the rab GTPase that functions in this trafficking step (Cooper et al. 2006), and causes accumulation of transport vesicles by inhibiting vesicle docking and/or fusion (Gitler and Shorter 2007). In mammalian cells, α-synuclein perturbs ER–Golgi trafficking as well (Gosavi et al. 2002), and PD-causing mutations increase this effect (Thayanidhi et al. 2010). α-Synuclein induces aggregation of rab proteins in yeast (Soper et al. 2011), triggering defects in endosomal trafficking. Mutant A30P α-synuclein interacts with rab3a, rab5, and rab8, potentially compromising synaptic vesicle trafficking, endocytosis, and α-synuclein transport (Dalfo et al. 2004). Additionally, abnormal binding of α-synuclein to rab3a in multiple system atrophy was observed (Dalfo and Ferrer 2005).

Dopamine Synthesis and Transport

α-Synuclein inhibits dopamine synthesis by inhibiting the expression and activity of TH (Masliah et al. 2000; Kirik et al. 2002; Perez et al. 2002; Baptista et al. 2003; Yu et al. 2004), likely by reducing the phosphorylation state of TH and stabilizing dephosphorylated inactive TH (Perez et al. 2002; Peng et al. 2005; Lou et al. 2010; Wu et al. 2011). In agreement, aging-related increases in α-synuclein expression in the substantia nigra negatively correlate with the expression of TH (Chu and Kordower 2007). Moreover, α-synuclein affects the dopamine-transporting vesicular transporter VMAT2: Knockdown of α-synuclein increases the density of VMAT2 molecules per vesicle, whereas overexpression inhibits VMAT2 activity, interrupting dopamine homeostasis by causing increased cytosolic dopamine levels (Guo et al. 2008).

Neurotransmitter Release and Synaptic Plasticity

The presynaptic localization of α-synuclein, its interaction with synaptic vesicles (Maroteaux et al. 1988; Perrin et al. 2000) and synaptobrevin-2 (Burré et al. 2010), its SNARE-complex chaperoning activity (Burré et al. 2010), and its changes during periods of song-acquisition-related synaptic rearrangement in birds (George et al. 1995) strongly suggests that α-synuclein plays a role in neurotransmitter release and synaptic plasticity, although its precise function remains unclear. Yet, absence of α-synuclein in worms, flies, and yeast suggests that α-synuclein is not required for synaptic transmission or membrane trafficking in general. Knockout of α-, α/β-, α/γ-, or α/β/γ-synucleins does not induce morphological changes in the brain (Abeliovich et al. 2000; Chandra et al. 2004; Burré et al. 2010; Anwar et al. 2011), although changes in synapse structure (Greten-Harrison et al. 2010) and an impairment in survival have been reported in triple-knockout mice (Burré et al. 2010; Greten-Harrison et al. 2010), suggesting that synucleins contribute to the long-term operation of a neuron.

The effect of α-synuclein on neurotransmission and synaptic plasticity has been investigated both in knockout and in overexpressing conditions, where α-synuclein has been reported to both promote and inhibit neurotransmitter release or have no effect at all. Although some studies report that α-synuclein does not have

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an effect on neurotransmitter release (Chandra et al. 2004; Watson et al. 2009; Burré et al. 2010), other studies report that α-synuclein enhances synaptic transmission (Steidl et al. 2003; Liu et al. 2004; Gureviciene et al. 2007, 2009; Greten-Harrison et al. 2010; Anwar et al. 2011; Vargas et al. 2014) or decreases release (Abeliovich et al. 2000; Cabin et al. 2002; Yavich et al. 2004, 2006; Larsen et al. 2006; Senior et al. 2008; Greten-Harrison et al. 2010; Nemani et al. 2010; Wu et al. 2010). A recent study has reported an inhibitory effect of α-synuclein on synaptic-vesicle endocytosis during intense stimulation, but not under basal levels (Busch et al. 2014; Vargas et al. 2014). Whether the inconsistent results obtained for the effects of α-synuclein on neurotransmission and synaptic plasticity could be ascribed to the experimental models used and the investigated brain regions needs to be determined.

How does α-synuclein exert its effect on the neurotransmission machinery? Within the presynaptic terminal, α-synuclein is highly mobile, as shown by photobleaching experiments, and it disperses from synaptic vesicles upon stimulation (Fortin et al. 2005). α-Synuclein modulates the mobility of synaptic vesicles between presynaptic boutons and maintains the overall size of the recycling pools at individual synapses (Scott and Roy 2012). It also organizes into multimers at synapses, which cluster synaptic vesicles, thereby restricting their motility (Wang et al. 2014) and likely attenuating exo/endocytosis. Multimerization is triggered by membrane binding and mediates SNARE-complex chaperoning activity (Burré et al. 2014). Moreover, α-synuclein clusters synaptic-vesicle mimics in vitro, via binding to the vesicles and synaptobrevin-2, and thereby prevents fusion of synaptic-vesicle mimics with plasma-membrane mimics (Fig. 6) (Diao et al. 2013), suggesting that α-synuclein provides a buffer of synaptic vesicles without affecting neurotransmitter release itself. In vitro, α-synuclein specifically inhibits vesicle docking without interfering with the fusion process (Lai et al. 2014). Overexpression causes accumulation of docked vesicles at synapses and smaller readily releasable pools (RRPs) (Larsen et al. 2006). Thus, the effect of α-synuclein on neurotransmitter release is likely not mediated by directly affecting the release machinery, but by regulating vesicle pools within the presynaptic terminal.

CONCLUDING REMARKS

Although α-synuclein has gained prominence for its role in neurodegenerative diseases called synucleinopathies, much about its cellular function(s) remains unclear, and little is known about how α-synuclein becomes cytotoxic and causes neurodegeneration when it is mutated or overexpressed. Diverse studies have proposed myriad functions for α-synuclein at locations ranging from the nucleus to mitochondria and nerve terminals. Of these, the strongest evidence has accumulated for the localization of α-synuclein at nerve terminals, specifically on the highly curved membrane of synaptic vesicles. Misfolded α-synuclein forms oligomers and aggregates that are believed to be toxic, and recent studies have revealed propagation of misfolded α-synuclein between neurons. Nucleation and propagation of α-synuclein misfolding, whether from the cytosolic pool or from the membrane-bound pool, remains controversial. There is now substantial evidence for toxicity of α-synuclein oligomers, as well as evidence for either a benign or protective role of larger aggregates and, potentially, Lewy bodies. Which species of α-synuclein is transferred from neuron to neuron, and how it is released, is entirely unknown and is a subject of intense research. Understanding how α-synuclein localizes and functions in subcellular compartments will facilitate understanding of central questions, such as how α-synuclein misfolds, which species of α-synuclein are toxic, how these species are released and taken up by neurons, and how these species may nucleate new aggregates in a healthy cell. The subcellular localization-specific functions of α-synuclein, such as those at nerve terminals, increase the possibility that pathological aggregation and inclusion of α-synuclein into Lewy bodies depletes the protein from locations where it functions, which occurs in addition to the direct toxic effects of misfolded α-synuclein aggregates. Thus, these are exciting times in α-synuclein research,

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and much will likely be revealed about this fascinating molecule in the near future.

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