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Functional and Pathological Effects of α-Synuclein on Synaptic SNARE Complexes

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Abstract

α-Synuclein is an abundant protein at the neuronal synapse that has been implicated in Parkinson's disease for over 25 years and characterizes the hallmark pathology of a group of neurodegenerative diseases now known as the synucleinopathies. Physiologically, α-synuclein exists in an equilibrium between a synaptic vesicle membrane-bound α-helical multimer and a cytosolic largely unstructured monomer. Through its membrane-bound state, α-synuclein functions in neurotransmitter release by modulating several steps in the synaptic vesicle cycle, including synaptic vesicle clustering and docking, SNARE complex assembly, and homeostasis of synaptic vesicle pools. These functions have been ascribed to α-synuclein's interactions with the synaptic vesicle SNARE protein VAMP2/synaptobrevin-2, the synaptic vesicle-attached synapsins, and the synaptic vesicle membrane itself. How α-synuclein affects these processes, and whether disease is due to loss-of-function or gain-of-toxic-function of α-synuclein remains unclear. In this review, we provide an in-depth summary of the existing literature, discuss possible reasons for the discrepancies in the field, and propose a working model that reconciles the findings in the literature.

Keywords

membrane binding; neuronal survival; Parkinson's disease; synaptic vesicle; neurotransmitter release

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V.G. and J.B. prepared the overall outline of the proposal and generated the figures. V.G., J.A.B, L.K., and J.B. wrote the manuscript.

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Introduction

α-Synuclein is an abundant presynaptic protein that binds to synaptic vesicles and functions in neurotransmitter release by modulating synaptic vesicle pools and chaperoning SNARE complex assembly through its interaction with vesicle-associated membrane protein 2 (VAMP2)/synaptobrevin-2. Pathologically, increased levels as well as mutations in α-Synuclein are linked to Parkinson's disease and related synucleinopathies, and SNARE dysfunction is a common occurrence in synucleinopathies. Here, we discuss the subcellular pools and functional conformations of α-Synuclein, its binding modes on synaptic vesicles, its role in chaperoning SNARE complex assembly and clustering synaptic vesicles, how this activity affects neurotransmission and the long-term functioning of neurons, and finally, we examine the role of SNARE dysfunction in synucleinopathies.

α-Synuclein exists in different subcellular pools

α-Synuclein protein is comprised of 140 amino acids and is encoded by the *SCNA* gene. It was initially identified in a screen for synaptic vesicle proteins, and named for its synaptic and nuclear localizations,[1] though subsequent studies have demonstrated that in the brain, it is found mainly in the presynaptic terminal of neurons.[2–3] There has been increased interest in α-Synuclein since the turn of the century because of two key findings implicating it in human disease: (1) α-Synuclein is abundant in Lewy bodies in sporadic cases of Parkinson's Disease (PD),[4] and (2) the missense mutation A53T in α-Synuclein causes familial PD.[5] Subsequently, additional mutations in α-Synuclein, including A30G,[6] A30P,[7] E46K,[8] H50Q,[9] G51D,[10–11] A53V,[12] A53E,[13] T72M,[14] and E83Q[15] (Figure 1), as well as gene duplication and triplication,[16–18] have been implicated in familial PD. Furthermore, genome-wide association studies show that common variability in the SNCA locus is associated with PD risk.[19]

α-Synuclein is intrinsically disordered in that it does not have a fixed three-dimensional structure in aqueous solution[20–21] (Figure 2). The N-terminal domain is positively charged, lysine-rich, and includes a seven 11-residue repeat sequence that forms an amphipathic α-helix upon lipid-binding[22] and membrane-binding[23] (Figure 1 and 2). It also contains the non-amyloid-beta component (NAC) domain which was identified in Alzheimer's disease (AD) neuropathology[24] that is aggregation-prone.[25–26] The C-terminal domain is negatively charged and glutamate-rich,[27–28] and is involved in interactions with proteins, DNA, ions, polyamines and metals[29–34] (Figure 1). The C-terminal domain also contains multiple phosphorylation sites, including at tyrosine residues 125, 133 and 136 and serine residue 129, of which S129 has been associated with pathology.[35–37]

In its native state, α-Synuclein exists as a largely unstructured monomer in the cytosol.[38–39] Upon membrane binding, it undergoes a conformational change to adopt an α-helical structure (Figure 2). α-Synuclein interacts with lipid membranes by association with negatively charged, acidic phospholipids, and its conformation upon binding depends on membrane curvature. A single α-helix forms on lower curvature membranes,[40] while a broken helix forms on higher curvature membranes,[41–42] mediating its physiologically relevant association with synaptic vesicle membranes. Membrane-bound α-Synuclein forms

multimers, a conformation that clusters synaptic vesicles and promotes SNARE complex assembly[43–44] (Figure 3).

The equilibrium between the cytosolic and membrane-bound pool of α-Synuclein is highly relevant for pathological and physiological conditions, although the role of membrane binding in pathology is debated. Membrane binding of α-Synuclein has been shown to be protective against aggregation, whereas the soluble, cytosolic pool of α-Synuclein is prone to aggregation[45] (Figure 2). Notably, all disease-associated α-Synuclein missense mutations are in the membrane-binding region, with some shown to decrease lipid binding[46–49] (Figure 1). However, membrane binding has also been shown to induce aggregation and pathology.[50–53] In pathological conditions, α-Synuclein is found in oligomeric conformations with β-sheet secondary structure and in fibrillar conformations,[54–55] which are believed to drive the neurotoxicity leading to neuronal cell death and clinical manifestations of PD (Figure 2).

What are the reasons underlying the apparent contradictions about the role of α-Synuclein in membrane binding? Early reports demonstrated that in a cell-free system, α-Synuclein aggregation occurred in the membrane fraction but not in the cytosol fraction, and that addition of the cytosolic fraction accelerated aggregation.[50] Further, α-Synuclein oligomers induced by *in vitro* chemical crosslinking associated preferentially with lipid droplets and cell membranes in cell culture.[53] In contrast, in presence of monomeric α-Synuclein, membrane-binding inhibited α-Synuclein oligomerization.[56] Understanding the conformational state of α-Synuclein is important to reconciling these results. Follow-up studies showed that induction of the α-helical conformation of α-Synuclein, which is associated with membranes in physiological states, was strongly correlated with inhibition of aggregation and fibril formation.[57] To address the interplay of membrane binding and neuropathology directly, introduction of α-Synuclein mutations that reduce or block membrane binding led to increased aggregation *in situ*, and virus-mediated striatal expression of these mutants led to accelerated motor dysfunction and neurotoxicity of dopaminergic neurons *in vivo* compared to neurons expressing wildtype α-Synuclein.[45,58] Taken together, α-Synuclein membrane binding is protective in physiological states, but correlates with aggregation after pathological changes in α-Synuclein such as oligomerization have already occurred (Figure 2).

α-Synuclein functions on the synaptic vesicle membrane

In development, α-Synuclein is initially somatic and concentrates in presynaptic terminals over time.[59–62] Of all the synaptic proteins, it is one of the last to become enriched in synaptic terminals, suggesting a role in maintenance of synaptic connections rather than development of synapses. Localization of α-Synuclein in the mature brain is primarily presynaptic.[3] While there is also evidence for localization on mitochondria and other organelles including the nucleus, this occurs primarily in overexpression conditions.[63–65] α-Synuclein lacks a transmembrane domain, and in fractionation experiments, α-Synuclein dissociates easily from membranes and was therefore not present in early isolations of synaptic fractions.[66] However, in a follow-up study with more sensitive detection

techniques, α-Synuclein was detected both in isolated synaptosomes and on purified synaptic vesicles.[67]

How is synaptic vesicle binding mediated? Interactions at both the N-terminal and C-terminal domains of α-Synuclein are important. As discussed above, ionic interactions between negatively charged acidic lipid head groups and positively charged lysine residues in the N-terminal domain[68–70] induce a conformational change in α-Synuclein that is favorable for association with synaptic vesicles.[41] Certain post-translational modifications, including N-terminal acetylation of α-Synuclein, induce α-helicity and lead to an increase in its membrane binding.[71] In contrast, heteromerization of the N-terminal lipidbinding domain of α-Synuclein with those of β-synuclein or γ-synuclein reduces synaptic vesicle binding of α-Synuclein in a concentration-dependent manner.[72]

The C-terminal domain of α-Synuclein interacts with proteins, DNA, ions, polyamines, and metals.[29–34] C-terminal domain calcium-binding increases α-Synuclein-synaptic vesicle interactions and vesicle clustering at the synaptic terminal.[73] The C-terminal domain also binds to VAMP2/synaptobrevin-2,[74–75] and this interaction is important for synaptic vesicle clustering and SNARE complex assembly[45,58,74–77] which is discussed further below. Functional consequences of the other interactions remain unclear and require further studies.

α-Synuclein chaperones SNARE complex assembly and clusters synaptic vesicles

Despite the body of research concerning α-Synuclein's localization and binding activity at the presynaptic terminal, there is an ongoing debate over its physiological function. Neurotransmission requires a tightly coordinated sequence of events at the nerve terminal (reviewed in[78]), canonically involving (1) clustering of a reserve pool of synaptic vesicles near synapses which are crosslinked by synapsins or by liquid–liquid phase separation[79] and released upon phosphorylation of synapsin[80], (2) docking of synaptic vesicles at active zones[81] that constitute the readily releasable pool[82], (3) formation of the ternary synaptic SNARE complex, that is composed of syntaxin-1, SNAP-25, and VAMP2/synaptobrevin-2,[83] (4) fusion of synaptic vesicles with the presynaptic plasmamembrane following calcium influx,[84] and (5) disassembly of the SNARE complex mediated by SNAPs and NSF, and endosomal recycling of synaptic vesicles (Figure 3).[85] The readily releasable pool undergoes exocytosis first upon stimulation, and is replenished by the reserve pool of synaptic vesicles upon depletion in the setting of sustained activity.[86–89]

The synuclein genes have only been identified in vertebrates, suggesting that they are not required for neurotransmission. However, α-Synuclein has been shown to modulate several steps in the synaptic vesicle cycle. Specifically, it has been implicated in synaptic vesicle clustering,[44,77,90] synaptic vesicle docking and SNARE complex formation,[43,74] and recycling pool homeostasis[44,75–76,91] (Figure 3). These functions are mediated by α-Synuclein's interactions with membranes,[43,58,77] with VAMP2/synaptobrevin-2[74–75,77], and synapsins.[92–93]

How is α-Synuclein exerting these functions on a molecular level? α-Synuclein has been shown to chaperone SNARE complex assembly via binding to the SNARE protein VAMP2/synaptobrevin-2, where amino acids 96–140 of α-Synuclein interact with amino acids

1–28 of VAMP2/synaptobrevin-2.[74] However, another study found that the C-terminus, specifically amino acids 110–140, were not required for the synaptic function of α-Synuclein.[91] A subsequent study reconciled these differences, confirming the importance of the functional interaction between α-Synuclein and VAMP2/synaptobrevin-2, and demonstrated that amino acids 96–104 are required for binding to α-Synuclein.[75] In cultured neurons, visualization of fluorescently tagged α-Synuclein in photobleaching experiments showed a similar abundance of α-Synuclein per vesicle as VAMP2/synaptobrevin-2, and binding of recombinantly expressed α-Synuclein to VAMP2/synaptobrevin-2.[94] Taken together, these results suggest that α-Synuclein-V AMP2/synaptobrevin-2 binding is essential for the maintenance of synaptic vesicle function.[74–75]

While the interaction between α-Synuclein and VAMP2/synaptobrevin-2 is established, the direction of the effect of this interaction on SNARE complex assembly is debated. In an *in vitro* liposome fusion setting, α-Synuclein oligomers and monomers cooperatively inhibited SNARE-mediated vesicle membrane fusion by inducing liposome clustering.[95] Taken in light of previous findings showing that physiological α-Synuclein promotes SNARE complex formation while pathological oligomers have a neurotoxic effect, these results support the hypothesis that the SNARE complex attenuation may represent a neurotoxic gain-of-function by α-Synuclein oligomers. This interaction may furthermore alternately enhance or inhibit SNARE complex formation depending on the presence of phosphatidylserine and either v-SNARE or t-SNARE proteins on the membrane of interest.[96]

In contrast, other data indicate that α-Synuclein's synaptic function is mediated only by its membrane-binding domain in a concentration-dependent manner, rather than by its binding to VAMP2/synaptobrevin-2. Attenuation of SNARE-dependent lipid membrane mixing correlated positively with α-Synuclein concentrations *in vitro*, while *in vivo* experiments using C-terminally truncated and point mutated α-Synuclein demonstrated that this activity is reliant on α-Synuclein's membrane binding ability, rather than its VAMP2/synaptobrevin-2 binding.[97] However, another *in vitro* study found that large α-Synuclein oligomers preferentially bound to the N-terminal domain of VAMP2/synaptobrevin-2, just like the monomer, and prevented SNARE complex formation, while α-Synuclein monomers had a negligible effect.[98] These results may help explain why oligomers seem to have a neurotoxic effect on dopaminergic neurons, while the monomeric form seems to have no such effect. Another study found that α-Synuclein inhibited SNARE-mediated vesicle fusion through modulating properties of the lipid bilayer, acting to increase the membrane fusion energy barrier by a variety of lipid-interaction mechanisms such as thinning membranes and increasing membrane rigidity without a direct interaction between α-Synuclein and any of the SNARE proteins.[99] Supportively, α-Synuclein was found to block SNARE complex formation without directly binding to SNARE proteins by buffering arachidonic acid, which has been shown to promote SNARE complex formation and decrease its availability to SNARE proteins.[100] While these disparate experimental findings seem contradictory, many of these studies were done *in vitro*, using purified proteins and liposomes at non-physiological concentrations. The applicability of these findings to neurons remains unclear.

Importantly, the effects of α-Synuclein on SNARE complex formation and synaptic vesicle clustering are mediated by synaptic vesicle membrane-bound α-Synuclein, and specifically by α-helical α-Synuclein multimers which assemble upon membrane binding.[43–44] These data support a model where synaptic vesicle clustering could be induced by the interaction of α-Synuclein multimers with VAMP2/synaptobrevin-2 on neighboring vesicles.[44,75] Simultaneous binding of α-Synuclein to VAMP2/synaptobrevin-2 and synaptic vesicle phospholipids has been shown to trigger clustering of synaptic vesicles.[44,77] Supportively, electron microscopy and cryoelectron tomography demonstrated that deletion of synucleins decreased the inter-linking of synaptic vesicles, and increased vesicle tethering to the active zone,[101] suggesting that α-Synuclein may modulate pools of synaptic vesicles at the synapse in part through synaptic vesicle clustering (Figure 3). Overexpression of α-Synuclein in a range predicted for gene multiplication in humans reduced the size of the reserve pool and the number of docked vesicles, suggesting that increased expression without overt toxicity leads to a physiologic defect in synaptic vesicle recycling.[91] Antibody-mediated disruption of α-Synuclein lead to a dose-dependent loss of synaptic vesicles in the reserve pool, as well as depletion of the docked readily releasable pool.[90] Taken together, these results suggest that α-Synuclein regulates vesicle recycling and docking in a dose-dependent manner, as both α-Synuclein loss-of-function and gain-of-function can impair vesicle recycling.

Synaptic vesicle clustering incorporates further interactions with additional proteins, including synapsins. Canonically, synapsin is essential for synaptic vesicle clustering as antibody-mediated blockade of synapsin function depletes the reserve pool while leaving the readily releasable pool intact.[86] Using synapsin triple-knockout mice, it was shown that synapsins may enhance clustering by targeting α-Synuclein to synapses, and that this interaction is also required for α-Synuclein-mediated effects on endocytosis.[92] Upon stimulation, synapsins dissociate from synaptic vesicles and disperse, followed by reclustering after the cessation of synaptic activity.[102] In a similar manner, α-Synuclein is highly mobile at the synapse, and disperses from the nerve terminal in response to activity and following exocytosis.[103] Synapsin regulates themobility of synaptic vesicle pools,[104] and further, a synapsin liquid phase has been shown to mediate synaptic vesicle clustering.[105–106] This liquid phase recruits α-Synuclein,[107] with a concentration-dependent effect on synaptic vesicle clustering and requiring a specific ratio of α-Synuclein and synapsin-1.[93]

Overall, a model emerges in which α-Synuclein induces synaptic vesicle clustering and crosslinking through a combination of lipid membrane and VAMP2/synaptobrevin-2 binding, which then reduces SNARE complex formation acutely by decreasing the number of synaptic vesicles available for docking at the presynaptic membrane. In the long term or during sustained activity, however, this activity of α-Synuclein may indirectly promote SNARE complex formation by providing a large reserve pool of vesicles, allowing for sustained SNARE complex formation and transmitter release. This model is supported by the activity-dependence of SNARE complex assembly in synuclein knockout mice.[74]

More research is required to fully characterize the nature and function of α-Synuclein-SNARE interactions and their effect on synaptic vesicle clustering and neurotransmitter release. Currently, this functionality appears to depend on a wide variety of factors,

including but not limited to α-Synuclein and SNARE protein concentrations, α-Synuclein conformations, α-Synuclein post-translational modifications, lipid membrane structure and composition, and the experimental system used to study this interaction. The disparate and in places conflicting findings from the literature reviewed here may be explained by several factors. Foremost, because α-Synuclein is an intrinsically disordered protein, its concentration and local environment can greatly affect its structure and function. This is compounded by its ability to form both oligomers and aggregates, which may further alter its function, potentially leading to both gain- and loss-of-function. Many of the assays performed in recent studies are *in vitro* and may not accurately reflect α-Synuclein's physiological state. Similarly, overexpression studies may result in pathological oligomeric α-Synuclein species, which may obscure any functionally relevant readout. Therefore, future studies focusing on physiologically relevant settings with advances in microscopy are expected to improve our understanding of this interaction. As argued above, such future studies may be able to better accommodate the apparent conflicts in the literature.

α-Synuclein's SNARE complex chaperoning activity is important for the long-term functioning of the nerve terminal

One of the initial descriptions of α-Synuclein protein was in the zebra finch, termed synelfin, which showed changes in its gene expression during a critical song-learning period suggesting a role in synaptic plasticity.[22] Later it was shown that αβγ-synuclein knockout mice have an age- and activity-dependent reduction in synaptic SNARE complex levels, suggesting a role for synucleins in the long-term function of the nerve terminal.[74] The role of α-Synuclein in mediating these functions has been investigated using various models modulating α-Synuclein expression, and taken together, strongly suggest that α-Synuclein modulates synaptic function and neuronal survival.

Yet, the direction of α-Synuclein's effects is controversial. α-Synuclein has been reported to have (1) no effect on neurotransmitter release,[74,108] (2) to enhance synaptic transmission,[109–113] or (3) to inhibit synaptic transmission.[112,114–121] The cumulative evidence indicates that α-Synuclein plays a *direct* role in regulating synaptic vesicle pools in the presynapse, as detailed in the previous section, with downstream, *indirect* effects on neurotransmission itself depending on the model system and conditions interrogated. Indeed, given the absence of α-Synuclein in invertebrates and mild phenotypes in knockout models, α-Synuclein is not required for neurotransmission in general, nor dopaminergic neurotransmission specifically.[122]

Here, we will focus on knockout studies to exclude the confounding effect of potential toxicity from overexpression studies. The majority of studies suggest an inhibitory role on neurotransmitter release at dopaminergic synapses. Synapses of α-Synuclein knockout mice showed a faster recovery of dopamine release following stimulation-induced depression, indicating an inhibitory effect on the recovery of dopamine release.[114] Of note, these mice had reduced levels of striatal dopaminewithout affecting the density of projections or dopamine reuptake, suggesting that the diminished recovery from depression observed in the knockout mice is due to a reduction of the readily releasable pool of dopamine-containing synaptic vesicles.[114] In agreement, α-Synuclein knockout mice demonstrated

strong facilitation of dopamine release with repeated stimulation that was not seen in wild-type mice, and deletion of all three members of the synuclein family showed greater dopamine release in dorsal striatal axons but not ventral striatal axons.[121] However, studies of hippocampal synapses in α-synuclein knockout and αβγ-knockout mice showed either synaptic facilitation,[109–113] or no effect at all.[74,108]

One explanation for these discrepancies is that α-synuclein may modulate synaptic function in an activity-dependent manner. Using αβγ-synuclein knockout mice, synuclein was shown to facilitate dopaminergic neurotransmission during bursts of activity separated by short intervals, and to depress neurotransmission during bursts of activity separated by long intervals.[123] However, it remains puzzling that multiple lines of evidence suggest that there is no effect of synuclein knockout on glutamatergic neurotransmission.[74,108,122] As it stands, there is no consensus for a mechanism on how α-synuclein directly acts on neurotransmitter release, in part due to the different regions interrogated, the interrogation of single synapses versus field recordings, the different stimulation protocols used, and the use of constitutive knockouts which may lead to compensation artifacts. Further experiments evaluating acute knockdown in different brain regions may be beneficial for clarification.

What is the role of α-synuclein over age and for neuronal survival? α-Synuclein knockouts have a reduction in dopaminergic neurons in the substantia nigra in some models,[124] especially with aging,[125] but these findings are not recapitulated in other α-synuclein knockouts.[114–115] However, analysis of mice of ages ranging from only a few months to less than a year may have hampered detection of neurodegeneration, in addition to potential compensatory changes in constitutive knockout animals. Of interest, α-synuclein knockout mice are resistant to MPTP-induced dopaminergic neurodegeneration,[126–128] suggesting that the normal function of α-synuclein is important to dopaminergic neuron viability and vulnerability in particular. Dopamine is thought to stabilize α-synuclein protofibrils and thus facilitate the selective degeneration of these neurons.[129] Indeed, overexpression of α-synuclein in chromaffin cells and a pheochromocytoma cell line led to elevated catechol concentrations, and this effect was even more pronounced in presence of overexpressed pathogenic A30P and A53T α-synuclein.[130] Thus, the toxic effects of α-synuclein likely rely on interactions with specific target neurons.

Importantly, constitutive removal of α-synuclein may lead to compensation over development, as knockdown of α-synuclein using virus-mediated expression of shRNA in the substantia nigra of adult animals led to rapid neuron loss beyond a certain threshold of reduced expression in both rodents and non-human primates.[131–134] This effect was not seen in the hippocampus, although the degree of knockdown may not have been sufficient.[135] Taken together, these results suggest that dopaminergic neurons are uniquely sensitive, and that while constitutive α-synuclein knockout leads to neuron loss in some paradigms, acute knockdown experiments demonstrate that α-synuclein is important for neuronal survival.

SNARE dysfunction in synucleinopathies

The hallmark of PD pathology is α-synuclein misfolding and aggregation and loss of dopaminergic neurons in the substantia nigra.[136] Yet, how alterations in SNARE proteins affect the molecular mechanisms in PD remains unknown. In cerebral cortex brain

homogenates from PD patients, a two-fold decrease in SNARE complex assembly was observed.[137] In visual cortex tissue from patients with dementia with Lewy bodies (DLB), significant reductions in syntaxin-1 and SNAP-25 were found.[138] This dysfunction may be clinically relevant, as in Parkinson's disease dementia (PDD) and DLB, decreased levels of VAMP2/synaptobrevin-2 correlated with duration of dementia.[139] In the striatum of a transgenic mouse model of PD, accumulation of α-synuclein in the synapses was accompanied by an age-dependent redistribution of the SNARE proteins SNAP-25, syntaxin-1 and VAMP2/synaptobrevin-2, as well as a reduction in dopamine release.[140] These results suggest a correlation between synaptic SNARE proteins and the pathogenesis of synucleinopathies. The vulnerability of dopaminergic neurons in these diseases may be due to the unique properties of their synaptic vesicle cycling. There was a higher rate of stimulus-induced endocytosis of the vesicular monoamine transport VMAT2 compared to the glutamate transporter VGLUT1 suggesting a cell-specific synaptic vesicle recycling mechanism in dopaminergic neurons to sustain high rates of release.[91]

Altered SNARE complex levels have been directly linked to neurodegeneration. Lack of the SNAP-25 chaperone CSPα triggers SNARE complex formation defects which results in neurodegeneration.[141–142] These deficits were rescued by expression of α-synuclein.[74,143] Conversely, in the presence of α-synuclein aggregates, viral delivery of CSPα rescued the impaired synaptic vesicle recycling in a pheochromocytoma cell line, reduced synaptic α-synuclein aggregates, and restored normal dopamine release in mice overexpressing α-synuclein 1-120.[144] SNARE complexes have also been proposed to mediate pathologically aggregated α-synuclein secretion via lysosomal exocytosis,[145] which may contribute to the hypothesized cell-to-cell transmission of pathological aggregates. In addition, the SNARE complex-regulating protein Munc18-1/STXBP1 has been proposed to protect α-synuclein from aggregation.[146] Interestingly, mutations in STXBP1 cause STXBP1 encephalopathies in humans,[147–148] and dysfunction of some of the STXBP1 mutants have been linked to early onset parkinsonism,[149–150] with co-aggregation of mutant Munc18-1 and α-Synuclein as a potential underlying mechanism.[146]

In Alzheimer's disease (AD), postmortem brain tissue showed defects in synaptic SNARE complex assembly, although expression of individual proteins remained the same,[137] while other studies have shown reduced expression of individual SNARE proteins.[151–153] In a mouse model expressing human amyloid precursor protein and presenilin-1 double-transgene, SNARE complex levels were substantially reduced, mediated by Aβ oligomers binding to syntaxin-1 and blocking of SNARE complex assembly.[154] Aβ peptides have also been shown to disrupt interactions between VAMP2/synaptobrevin-2 and SNAP-25 in neuronal cells, and Aβ42 was found to compete with VAMP2/synaptobrevin-2 for binding to synaptophysin at synapses.[155]

Is there a link to α-Synuclein in AD? Interestingly, α-Synuclein co-pathology has been reported in more than half of all autopsy-confirmed AD cases,[156–158] and increased cerebrospinal fluid α-Synuclein levels were found in patients with or at risk of developing AD.[159–160] Aβ and α-Synuclein have been shown to co-immunoprecipitate in the brains of patients with AD/PD and in transgenic mice.[161] In mice, reducing endogenous α-Synuclein in an APP transgenic mouse model prevented the degeneration of cholinergic neurons and

decreased corresponding behavioral deficits.[162] However, in another study, overexpression of α-Synuclein in APP animals reduced Ab plaque deposition, but exacerbated deficits in spatial memory, increased extracellular Aβ oligomers, α-Synuclein oligomers, and exacerbated tau variants associated with AD.[163] Ablating α-Synuclein improved memory retention in spite of increased plaque burden, prevented premature mortality, decreased extracellular Aβ oligomers, and rescued postsynaptic marker deficits.[163] While these studies provide a possible link between SNARE complexes, α-Synuclein, and AD pathogenesis and progression, the molecular mechanisms on how α-Synuclein affects AD pathology remain unclear.

Altogether, there is accumulating evidence to suggest that there is a role for synaptic SNARE complex deficits in PD, DLB, and AD. With more evidence for a relationship between α-Synuclein and SNARE-dependent membrane fusion and/or SNARE complex formation, and with several studies showing SNARE dysfunction affecting disease pathology in AD, this leaves an exciting opportunity for further scientific advancements. It furthermore suggests that therapeutic strategies that aim at inhibiting aggregation of α-Synuclein or facilitating α-Synuclein clearance, such as small molecules and autophagy inhibitors,[164–172] may benefit from targeting SNARE proteins as well.

Perspectives

There is a clear link between α-Synuclein and SNARE complex assembly. This connection is both functionally important and disease-relevant. However, it remains unclear if lack of SNARE complex assembly in synucleinopathies is due to α-Synuclein aggregation, if SNARE dysfunction in the context of α-Synuclein aggregation is involved in disease pathogenesis, or if α-Synuclein aggregation itself is the major driver of pathology. Whether α-Synuclein pathology is due to toxic gain-of-function or loss-of-function is an ongoing debate in the field. Several studies suggest that the physiological functions and pathological activities of α-Synuclein are mediated by different regions of α-Synuclein. Disruptions in the physiological functions of α-Synuclein do not always lead to pathological aggregation and cell death. However, loss-of-function and toxic gain-of-function cannot be studied in isolation. To resolve this long-standing question in the field, both have to be studied in a physiologically relevant context.

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Abbreviations:

AD	Alzheimer’s disease
APP	amyloid precursor protein
CSPα	cysteine string protein α
DLB	dementia with Lewy Bodies

MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
NAC	non-amyloid-beta component
NSF	*N*-ethylmaleimide sensitive fusion protein
PD	Parkinson's disease
PDD	Parkinson's disease dementia
SNAP	soluble NSF-attachment protein
SNAP-25	synaptosomal-associated protein of 25 kDa
SNARE	soluble NSF-attachment protein receptor
VAMP2	vesicle-associated membrane protein 2
VGLUT1	vesicular glutamate transporter 1
VMAT2	vesicular monoamine transporter 2

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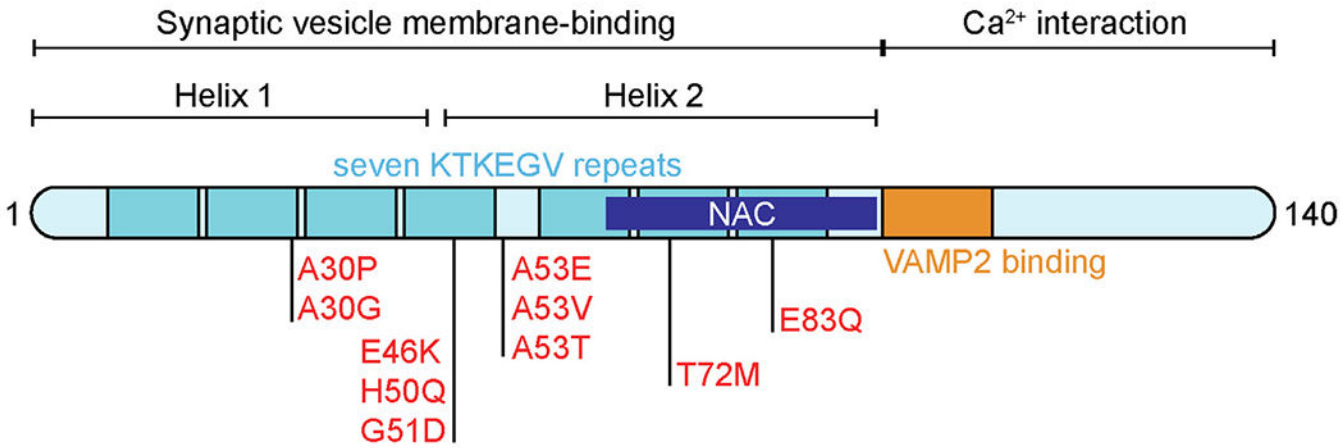

Figure 1.

Structure of α-synuclein. The N-terminal domain is comprised of seven imperfect KTKEGV repeats that form two amphipathic α-helices upon binding to synaptic vesicle membranes. This region also contains the aggregation-prone NAC domain, as well as all disease-linked mutations. The C-terminal domain of α-synuclein is not involved in membrane binding and mediates interactions with calcium as well as the SNARE protein VAMP2/synaptobrevin-2.

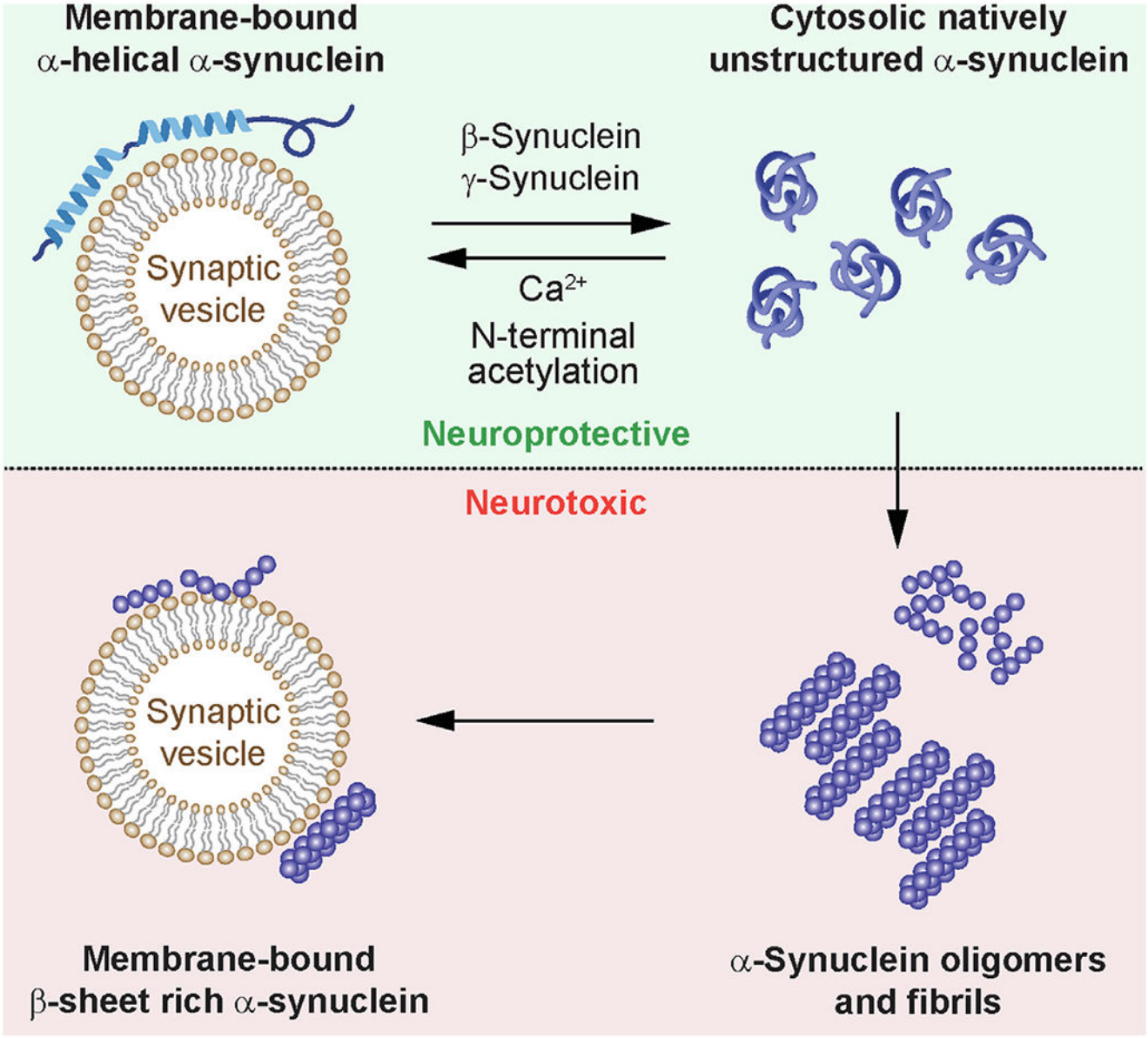

Figure 2.

Implications of membrane binding of α-synuclein for function and disease. Under physiological conditions, α-synuclein exists in equilibrium between a membrane-bound α-helical conformation on synaptic vesicles and a natively unstructured state in the cytosol. Membrane binding is facilitated by calcium and N-terminal acetylation, whereas the cytosolic pool is increased in presence of β-synuclein or γ-synuclein. α-Synuclein oligomers and fibrils form from the unstructured cytosolic state, leading to neuropathology. Membrane binding of α-synuclein is neuroprotective in its α-helical multimeric state, while association of β-sheet rich oligomeric or fibrillar α-synuclein with synaptic vesicles is linked to neurotoxicity.

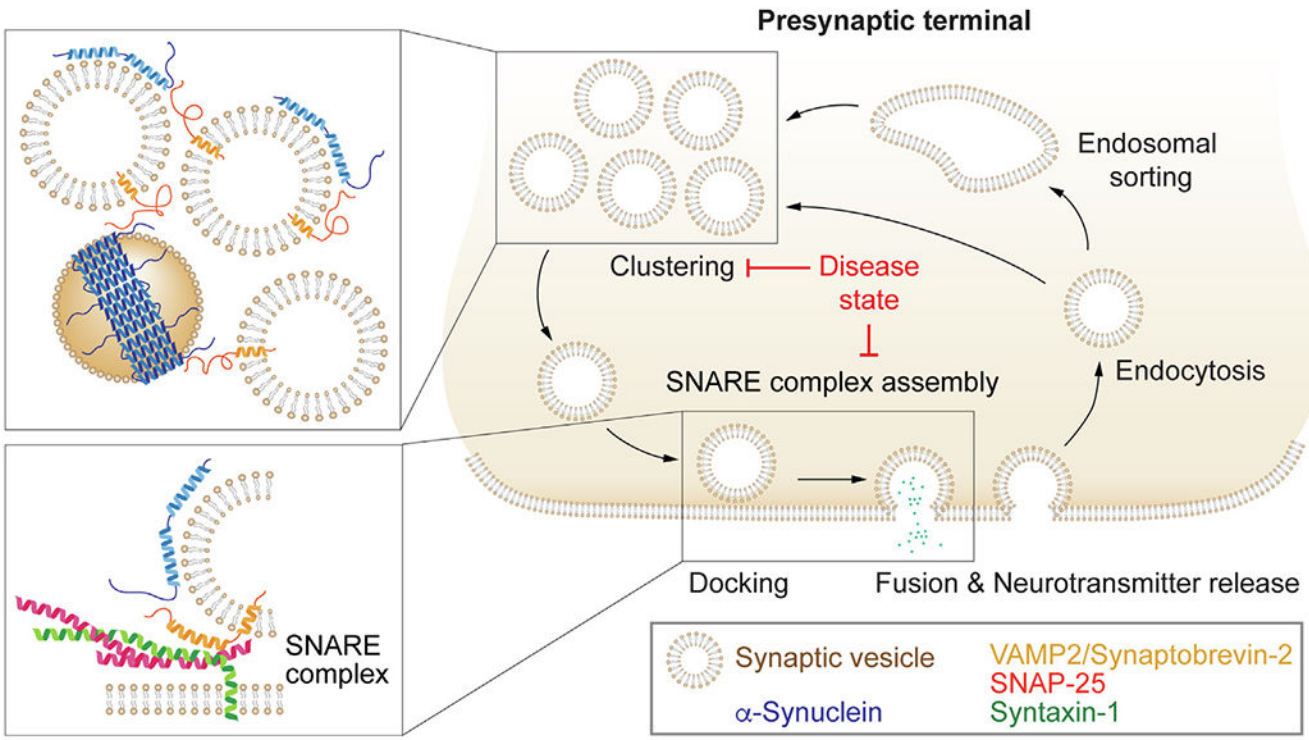

Figure 3.

SNARE-dependent effects of α-synuclein on the synaptic vesicle cycle. Upon initiation of an action potential in the presynaptic terminal, synaptic vesicles docked to the plasma membrane fuse to release neurotransmitters to propagate the signal to the next neuron. Synaptic vesicle fusion is mediated by formation of the synaptic SNARE complex, composed of syntaxin-1 and SNAP-25 on the presynaptic plasma membrane, and VAMP2/synaptobrevin-2 on the synaptic vesicle. Following fusion, synaptic vesicle constituents are retrieved via endocytosis. Simultaneous binding of α-synuclein multimers to synaptic vesicle membranes and the synaptic vesicle protein VAMP2/synaptobrevin-2 clusters synaptic vesicles (top inset) and chaperones SNARE complex assembly (bottom inset). In diseased states, pathological α-synuclein or lack of physiologically functional α-synuclein due to recruitment into pathological α-synuclein aggregates results in impaired or dysfunctional synaptic vesicle clustering and reduced SNARE complex assembly. This imbalance in the synaptic vesicle cycle affects long-term neuron function and survival.