

# The many faces of $\alpha$ -synuclein: from structure and toxicity to therapeutic target

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Abstract | Disorders characterized by  $\alpha$ -synuclein ( $\alpha$ -syn) accumulation, Lewy body formation and parkinsonism (and in some cases dementia) are collectively known as Lewy body diseases. The molecular mechanism (or mechanisms) through which  $\alpha$ -syn abnormally accumulates and contributes to neurodegeneration in these disorders remains unknown. Here, we provide an overview of current knowledge and prevailing hypotheses regarding the conformational, oligomerization and aggregation states of  $\alpha$ -syn and their role in regulating  $\alpha$ -syn function in health and disease. Understanding the nature of the various  $\alpha$ -syn structures, how they are formed and their relative contributions to  $\alpha$ -syn-mediated toxicity may inform future studies aiming to develop therapeutic prevention and intervention.

Lewy body diseases (LBDs) are a heterogeneous group of disorders that include Parkinson's disease (PD), PD with dementia (PDD) and dementia with Lewy bodies (DLB)<sup>1</sup>. Together, these diseases affect over 5 million people worldwide. LBDs are mainly characterized pathologically by the progressive accumulation of the presynaptic protein  $\alpha$ -synuclein ( $\alpha$ -syn) — hence they are often referred to as synucleinopathies — and by the degeneration of neocortical, limbic and nigral-striato circuitries<sup>2,3</sup>. A pathogenic role for  $\alpha$ -syn in these disorders is supported by various genetic data. Multiplications of the gene encoding  $\alpha$ -syn (*SNCA*)<sup>4</sup> and various point mutations in this gene (A53T, A30P and E46K)<sup>5–7</sup> result in dominant familial parkinsonism. Moreover, certain polymorphisms in *SNCA* are major risk factors for sporadic PD<sup>8</sup>.

An increasing body of evidence from animal models as well as data from genetic, biochemical and biophysical studies support the hypothesis that the processes of  $\alpha$ -syn oligomerization<sup>9,10</sup> and fibril growth<sup>11,12</sup> have central roles in the pathogenesis of PD and other synucleinopathies<sup>13</sup>. In addition, it is possible that the  $\alpha$ -syn monomers might also have a role in synucleinopathies by their displacement from their physiological location, resulting in a loss of cellular function, or by disrupting the activity of other molecular or signalling pathways<sup>14</sup>. Nevertheless, the molecular mechanisms by which  $\alpha$ -syn aggregation contributes to neurodegeneration,

the nature of the toxic forms of  $\alpha$ -syn and the cellular pathways that are affected by  $\alpha$ -syn remain unknown. Addressing these knowledge gaps will be crucial for understanding the molecular basis of synucleinopathies, developing tools to diagnose and monitor the progression of these diseases, and assessing the effectiveness of preventive and therapeutic strategies. Here, we provide an overview of the current state of knowledge regarding the role of  $\alpha$ -syn aggregation states in regulating  $\alpha$ -syn function under physiological and pathophysiological conditions. We also discuss the implications of these findings for therapeutic interventions.

## Physiological function of $\alpha$ -synuclein

Although the normal function (or functions) of  $\alpha$ -syn remains unknown, its localization at presynaptic terminals<sup>15–17</sup> (FIG. 1 a,b), its association with the distal reserve pool of synaptic vesicles<sup>18–20</sup> and the deficiencies in synaptic transmissions observed in response to knockdown or overexpression of  $\alpha$ -syn suggest that  $\alpha$ -syn has a role in the regulation of neurotransmitter release, synaptic function and plasticity (FIG. 1 c).

*Snca*-knockout mice exhibit an impairment in hippocampal synaptic responses to prolonged trains of high-frequency stimulation that deplete the docked and reserve pool of synaptic vesicles, as well as impairments in replenishment of docked pools from the reserve pool, indicating that  $\alpha$ -syn may control the refilling and the

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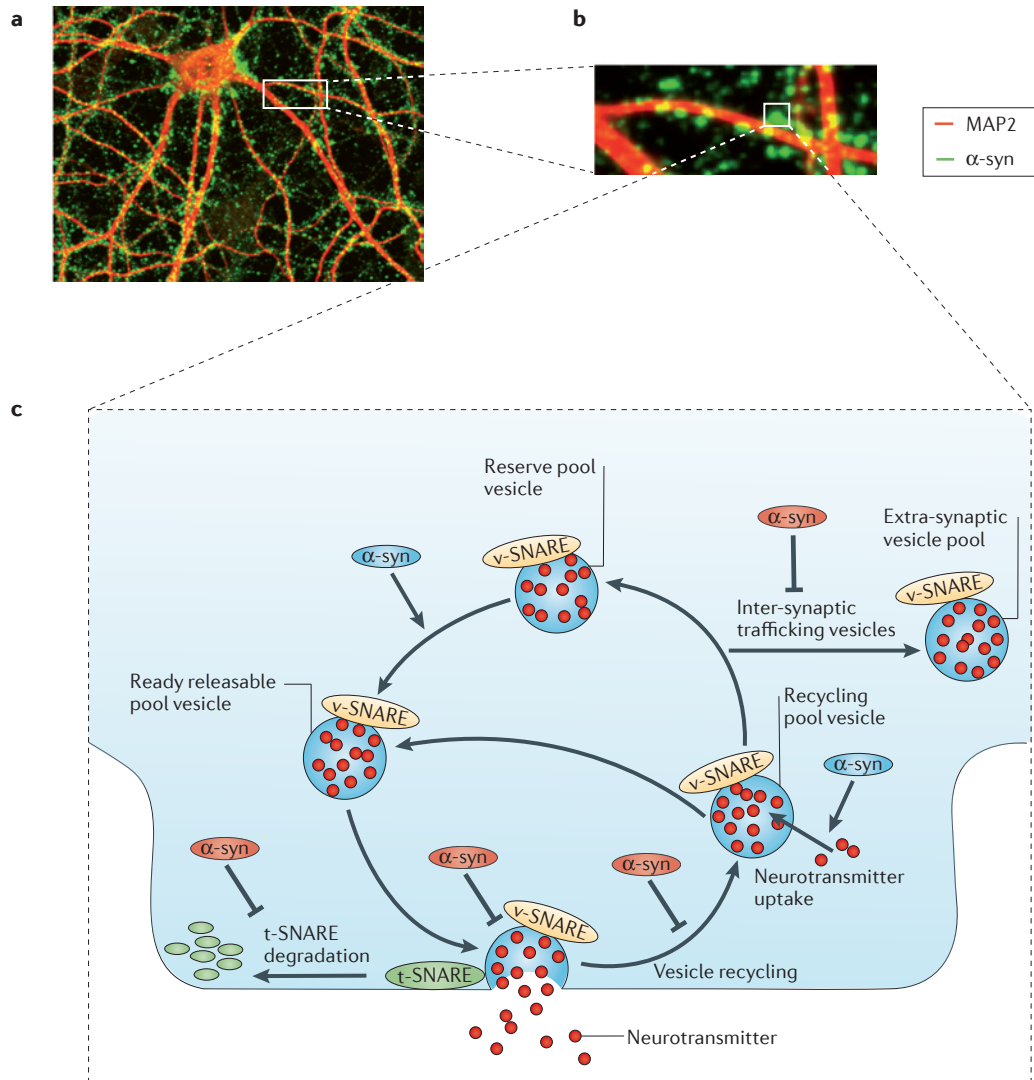
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**Lewy body**

An intraneuronal globular inclusion composed primarily of  $\alpha$ -synuclein fibrils. Lewy bodies are characteristically found in Parkinson's disease, although they are also detectable in other neurodegenerative diseases, such as dementia with Lewy bodies.

**Fibril**

Mature fibrils are characterized by the following characteristics: a cross- $\beta$ -sheet X-ray fibre diffraction pattern;  $\beta$ -sheet-rich circular dichroism and Fourier transform infrared spectroscopy (FTIR) spectra; binding to Congo red and Thioflavin-T/S; and a characteristic filamentous morphology (8–12 nm in diameter and > 1  $\mu$ m in length), as revealed by atomic force microscope and transmission electron microscope imaging.  $\alpha$ -synuclein can form fibrils of diverse morphologies depending on the solution conditions.



**Figure 1 | Functional properties of  $\alpha$ -synuclein.** **a, b** | Wide field (**a**) and magnified (**b**) images of cultured cortical neurons from a postnatal day 1 wild-type mouse. A neuronal dendrite (as revealed by MAP2 immunostaining; red) is shown opposed to  $\alpha$ -synuclein ( $\alpha$ -syn)-positive presynaptic densities (green), indicating that  $\alpha$ -syn is located in presynaptic terminals. **c** | This schematic highlights the roles of  $\alpha$ -syn at the presynaptic terminal in the regulation of vesicle trafficking and vesicle refilling ( $\alpha$ -syn; blue), as well as the interactions between target membrane-associated SNARE (t-SNARE) and vesicle-associated SNARE (v-SNARE) proteins and neurotransmitter release. Accumulation of  $\alpha$ -syn induces an impairment of neurotransmitter release, vesicle recycling and trafficking between synaptic buttons and influences the stability of t-SNARE complex assembly ( $\alpha$ -syn; red). Depletion of  $\alpha$ -syn induces an impairment of vesicle trafficking between the reserve pool and the ready releasable pool and a deficiency in vesicle refilling and neurotransmitter uptake (not shown).

trafficking of synaptic vesicles from the reserve pool to the site of synaptic vesicle release<sup>21,22</sup>. Moreover, depletion of  $\alpha$ -syn, through the use of antisense oligonucleotides, induces a decrease in the availability of reserve synaptic vesicle pool in primary cultured hippocampal neurons<sup>23</sup>.

Transgenic mice overexpressing human  $\alpha$ -syn exhibit impairment in synaptic vesicle exocytosis and a reduction in neurotransmitter release<sup>24–26</sup>. Similar effects have been observed after  $\alpha$ -syn overexpression in genetic rodent models of PD<sup>27,28</sup> and in the PC12 stable cell line<sup>29</sup>. At the ultrastructural level, overexpression of  $\alpha$ -syn induces a decrease in readily releasable vesicles<sup>27</sup> and

affects the recycling of synaptic vesicles following endocytosis, inducing a reduction in the size of the synaptic vesicle recycling pool<sup>26</sup>. Moreover, excess  $\alpha$ -syn induces a reduction in dopamine reuptake in dopaminergic terminals<sup>28</sup> and inhibits intersynaptic trafficking of vesicles, leading to a smaller reserve pool of vesicles<sup>30</sup>.

The possible role of  $\alpha$ -syn in regulating synaptic homeostasis is not exclusively related to its direct interaction with synaptic vesicles.  $\alpha$ -syn interacts with synaptic proteins controlling vesicle exocytosis, such as phospholipase D2 (REF. 31) and the family of RAB small GTPases<sup>32</sup>. Recent studies reported that  $\alpha$ -syn can act as

**SNARE**

Soluble NSF (N-ethylmaleimide-sensitive factor) attachment protein (SNAP) receptor.

**Oligomer**

Oligomers comprise a number of smaller, identical units (monomers). In the context of  $\alpha$ -synuclein oligomers, this term encompasses a wide range of species, ranging from low-molecular-weight species (including dimers, trimers and tetramers) to high-molecular-weight species (such as spherical, chain-like and annular structures).

a chaperone protein for the presynaptic SNARE protein complex by controlling the degradation and affecting the assembly, maintenance and distribution of this complex (FIG. 1c), which is directly implicated in the release of neurotransmitters, including dopamine<sup>33</sup>. Together, these observations indicate that  $\alpha$ -syn has an important role in the trafficking of synaptic vesicles and in the regulation of vesicle exocytosis, and may contribute to more subtle regulatory phenomena by controlling synaptic homeostasis-associated proteins.

The fact that the individual synuclein knockouts ( $\alpha$ -,  $\beta$ - and  $\gamma$ -syn) are viable suggests that synucleins are not essential components of the neurotransmitter release machinery but may contribute to the long-term regulation and maintenance of nerve terminal function<sup>34</sup>. The neuroprotective effects of  $\alpha$ -syn against progressive neurodegeneration in mice deficient in cysteine-string protein- $\alpha$  (CSP $\alpha$ ; also known as DNAJC5)<sup>35</sup> strongly suggest that its functional properties become more prominent or essential under conditions of stress. The neuroprotective function of  $\alpha$ -syn appears to be mediated by its ability to bind to membranes and vesicles, as the A30P  $\alpha$ -syn mutant, which is deficient in membrane binding, failed to show protection in CSP $\alpha$  gene-knockout mice.

**Structure of  $\alpha$ -synuclein**

Because a protein's sequence and structure are linked to its function, a concerted effort has been made to characterize the sequence and structural determinants that govern the cellular properties of  $\alpha$ -syn and its aberrant behaviour in PD and other synucleinopathies.  $\alpha$ -synuclein is a 14 kDa protein (140 amino acids;  $pK_a$  of 4.7)<sup>36</sup> that is characterized by an amphipathic lysine-rich amino terminus, which has a crucial role in modulating its interactions with membranes, and a disordered, acidic carboxy-terminal tail, which has been implicated in regulating its nuclear localization and interactions with metals, small molecules and proteins<sup>37,38</sup> (FIG. 2a). The central region of  $\alpha$ -syn was first purified from amyloid plaques in patients with Alzheimer's disease (AD)<sup>36,39</sup>. It contains a highly hydrophobic motif that comprises amino acid residues 65–90 and is known as the non-amyloid- $\beta$  component of AD amyloid plaques (NAC)<sup>36,40,41</sup> (FIG. 2a). The NAC region is indispensable for  $\alpha$ -syn aggregation; the deletion of large segments within this motif greatly diminished  $\alpha$ -syn oligomerization and fibrillogenesis *in vitro*<sup>42,43</sup> and in a cell-based assay<sup>44</sup>.

Studies by several groups using different biophysical methods (for example, NMR, light scattering and circular dichroism) consistently showed that  $\alpha$ -syn purified from *Escherichia coli* under native or denaturing conditions exists predominantly as stable unfolded monomers<sup>38,45</sup>. When  $\alpha$ -syn was extracted from patients diagnosed with LBD and age-matched controls and evaluated by non-denaturing gels or size exclusion chromatography (SEC) columns,  $\alpha$ -syn monomers migrated as 57–60 kDa proteins, but in denaturing gels they migrated as 14 kDa proteins<sup>42,44</sup> (FIG. 2b). Initially, the apparent size of  $\alpha$ -syn in native gels and SEC led many researchers to propose that this protein exists as a stable oligomer, but subsequent

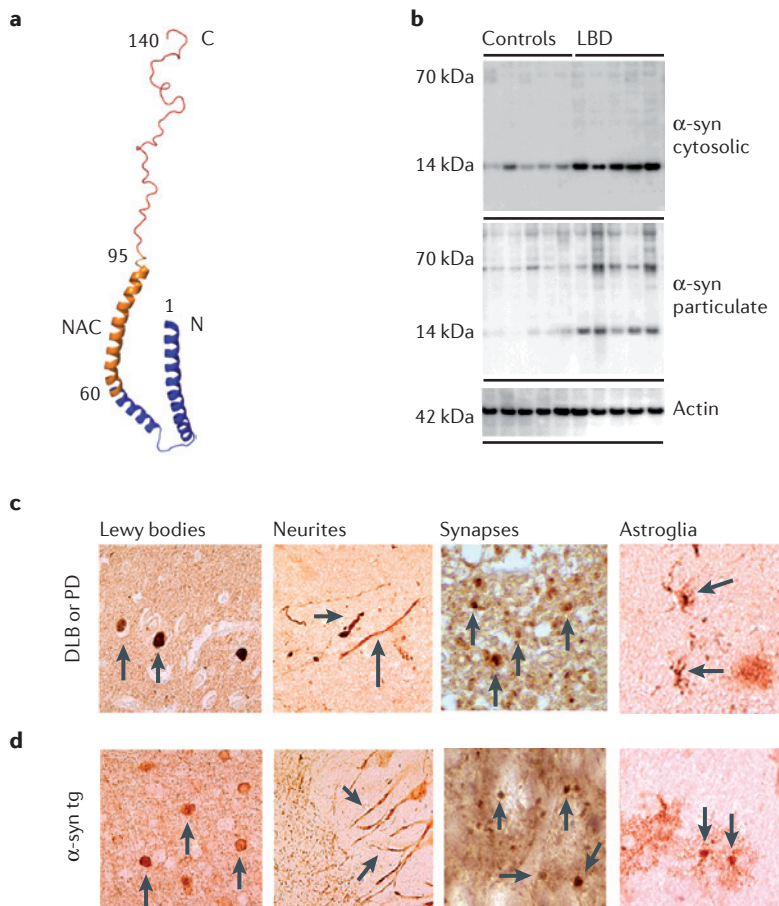
detailed biophysical studies demonstrated that the larger than expected size is mainly due to the fact that monomeric  $\alpha$ -syn adopts an unfolded, extended conformation<sup>45,46</sup>, which results in a larger than expected hydrodynamic radius (BOX 1).

The apparently multifunctional properties of  $\alpha$ -syn may lie in its conformational flexibility, which may allow the protein to adopt different conformations upon interacting with biological membranes of different compositions, other proteins or protein complexes<sup>47,48</sup>. It is well established that  $\alpha$ -syn adopts an  $\alpha$ -helical conformation upon binding to synthetic or biological membranes *in vitro*<sup>38</sup>. However, very little is known about the conformational state (or states) of  $\alpha$ -syn in the different compartments of the living cell, and there is a lack of direct evidence for a functional role of native  $\alpha$ -syn oligomers in biological membranes. It is conceivable that native  $\alpha$ -syn exists in equilibrium between different conformational and/or oligomeric states. Several factors, including oxidative stress<sup>49</sup>, post-translational modifications<sup>50,51</sup>, proteolysis<sup>52,53</sup> and the concentrations of fatty acids<sup>54–56</sup>, phospholipids and metal ions<sup>11,49</sup>, were shown to induce and/or modulate  $\alpha$ -syn structure and oligomerization *in vitro*, and these factors may influence this equilibrium between the monomer and oligomer state *in vivo*. Together, these studies suggest that  $\alpha$ -syn exists predominantly as a monomer, but do not rule out the possibility that it can form a stable multimer and/or adopt different structures under specific stress-induced conditions or upon interaction with other proteins, specific ligands, lipids and/or biological membranes. Understanding the molecular determinants that regulate the conformational and oligomeric states of native  $\alpha$ -syn and the structural basis of its toxicity will be crucial for our ability to generate and test different mechanistic models and hypotheses of  $\alpha$ -syn function in health and disease. Gaining this knowledge will also have important implications for the development of therapeutic strategies based on targeting  $\alpha$ -syn, as discussed in the following section.

 **$\alpha$ -synuclein and disease**

**Paths to increased expression and accumulation of  $\alpha$ -synuclein.** PD, DLB and other LBDs show accumulation and redistribution of  $\alpha$ -syn in various brain regions and cellular populations. These changes in the nature and localization of  $\alpha$ -syn may have pathogenic roles in these disorders (FIG. 2c) and can be reproduced in  $\alpha$ -syn transgenic animal models<sup>1,3,57–59</sup> (FIG. 2d).

The levels of  $\alpha$ -syn in the CNS depend on the balance between the rates of  $\alpha$ -syn synthesis, aggregation and clearance<sup>60</sup> (FIG. 3a,b). An imbalance between these mechanisms, caused by dysfunction of one or more of these pathways, can result in abnormal levels of  $\alpha$ -syn that might favour the formation and/or accumulation of oligomeric and fibrillar species, which may be toxic. Indeed, in some familial forms of parkinsonism, multiplication of *SNCA* results in increased accumulation of  $\alpha$ -syn because of increased protein expression<sup>4</sup>, whereas in others, *SNCA* mutations enhance the propensity of  $\alpha$ -syn to aggregate<sup>9</sup> (FIG. 3a). A genome-wide association



**Figure 2 | Biochemical structure of  $\alpha$ -synuclein and its pathological distribution in Parkinson's disease, and a mouse model of Lewy body disease.** **a** | Schematic representation of micelle-bound  $\alpha$ -synuclein ( $\alpha$ -syn; Protein Data Bank ID: 1XQ8)<sup>37</sup>. The N-terminal region, the non-amyloid- $\beta$  component of Alzheimer's disease amyloid plaques (NAC) region and the C-terminal part are coloured blue, orange and red, respectively. Numbers refer to amino acid residues flanking the different regions. **b** | Western blot identifying  $\alpha$ -syn in brain homogenates from control and Lewy body disease (LBD) cases that were divided into cytosolic and particulate fractions.  $\alpha$ -syn migrated to 57–60 kDa as well as to 14 kDa in the particulate but not cytosolic fraction owing to the different conformational states of the protein. **c,d** |  $\alpha$ -syn is present in Lewy bodies, neurites, synapses and astroglia in dementia with Lewy bodies (DLB) and Parkinson's disease (PD) and in transgenic (tg) mice overexpressing human wild-type  $\alpha$ -syn (known as PDGFB–human wild-type  $\alpha$ -syn mice), as indicated by arrows.

study (GWAS) showed that individuals with certain variations in the *SNCA* gene had a higher risk of PD<sup>61</sup>. One such polymorphism is known as Rep1, which occurs in the promoter region of *SNCA* and might increase the susceptibility to PD by increasing the expression of  $\alpha$ -syn<sup>62</sup>. Clearance of  $\alpha$ -syn monomers and aggregates occurs via direct proteolysis (for example, by neurosin or matrix metalloproteinase 9 (MMP9))<sup>63</sup>, binding to molecular chaperones (for example, heat shock proteins (HSPs))<sup>64</sup>, the proteasome<sup>65–67</sup> and autophagy (involving the activity of the lysosome)<sup>60,68–70</sup> (FIG. 3a). In sporadic forms of PD and DLB, failure of the autophagy pathways to eliminate oligomers might enhance  $\alpha$ -syn-mediated toxicity<sup>69</sup> (FIG. 3a). Chaperone-mediated autophagy<sup>68</sup> has been shown to be disrupted by oligomeric forms of wild-type and disease-associated mutant  $\alpha$ -syn. In PD

and DLB, the levels of key autophagy molecules such as ATG7, a ubiquitin-like modifier-activating enzyme, and mammalian target of rapamycin (mTOR), a serine/threonine protein kinase, are dysregulated<sup>71</sup>.

**Evidence for different conformers of  $\alpha$ -synuclein in disease.** There are a number of different  $\alpha$ -syn conformers, including oligomers, protofibrils and fibrils, that have been associated with the pathogenesis of LBDs. The fibrillar forms of  $\alpha$ -syn are detected mostly in Lewy bodies<sup>72,73</sup>, which are localized in the neuronal cell body. These intracellular structures are the neuropathological hallmark of PD and DLB (FIG. 2b) and are thought to reflect an attempt by the neurons to isolate and/or convert toxic  $\alpha$ -syn oligomers to fibrils, which are stable, less dynamic structures that exhibit reduced toxicity. Aggregates of  $\alpha$ -syn can also be observed in astroglial cells from cases of LBD<sup>74</sup>. The distribution of  $\alpha$ -syn pathology in human diseases can be recapitulated in  $\alpha$ -syn transgenic mice<sup>75</sup> (FIG. 2c).

Strong indirect evidence supports the existence of various oligomeric  $\alpha$ -syn species *in vivo* under pathophysiological conditions. SDS-resistant dimers as well as low- and high-molecular-weight oligomeric forms of  $\alpha$ -syn have been detected in diseased human brains<sup>10,76,77</sup> and in brains of transgenic animal models of synucleinopathies<sup>10,18,78</sup> that express wild-type or PD-associated mutant variants of  $\alpha$ -syn<sup>79</sup> (FIG. 2a). In contrast to fibrillar  $\alpha$ -syn, oligomeric aggregates are most likely to be located in axons (FIG. 1b) and presynaptic terminals, where they might damage synapses and dendrites<sup>25,26,28,80–82</sup>.

*In vitro*, several oligomeric species of different morphologies, including spherical, chain-like and annular oligomers, have been observed before  $\alpha$ -syn fibril formation<sup>83</sup> (FIG. 4). The various oligomeric species seem to exist in equilibrium with monomeric  $\alpha$ -syn and undergo a very slow conversion to fibrils in the absence of a high molar ratio of monomers to other species of  $\alpha$ -syn (FIG. 4). However, the relationships between the various  $\alpha$ -syn oligomeric species and mechanisms of the inter-conversion between these different oligomers remain poorly understood, although some studies suggest that the formation of ring-like oligomers is not on the pathway to amyloid formation. Of note, both *in vitro* and animal model studies show that three PD-linked *SNCA* mutations (A30P, E46K and A53T) accelerate  $\alpha$ -syn oligomerization, but only two of these (E46K and A53T) enhance fibrillization *in vitro* and *in vivo*<sup>9,42,84</sup>. Although the A30P mutation has been shown to result in enhanced  $\alpha$ -syn fibrillization *in vivo*, as illustrated by an autopsy case of a patient with this mutation who had extensive Lewy body pathology<sup>61</sup>, *in vitro* it exhibits reduced fibrillization compared with the wild-type protein and other mutants.

Biophysical and SEC studies suggest that  $\alpha$ -syn SDS-resistant oligomers from post-mortem human and transgenic animal brains can be in general divided into small (~2–5-mers), medium (~5–15-mers), and large (~15–150-mers) oligomers<sup>85,86</sup>. Spherical oligomers 2–6 nm in diameter may be the toxic forms of  $\alpha$ -syn, as they promote neuronal degeneration and abnormal calcium currents in cultured primary cortical neurons<sup>86</sup>.

Box 1 | The native state of  $\alpha$ -synuclein

The wide distribution and abundant expression of  $\alpha$ -synuclein ( $\alpha$ -syn) throughout the brain have been difficult to reconcile with its unfolded structure, which is expected to make the protein more susceptible to proteolysis and degradation. Thus, it was proposed that the oligomerization of  $\alpha$ -syn or its association with other proteins might represent mechanisms by which the protein may acquire different structures and functions *in vivo*. Two recent studies reported that native  $\alpha$ -syn exists as a folded tetramer<sup>126,127</sup>. The first study showed that  $\alpha$ -syn purified from mammalian cell lines or red blood cells exists as a stable  $\alpha$ -helical tetramer with an apparent size of 58–60 kDa, as was discerned by size exclusion chromatography (SEC), native PAGE (clear and blue-native PAGE) and sedimentation equilibrium studies<sup>126</sup>. The second study used NMR, chemical crosslinking and SEC, and indicated that  $\alpha$ -syn produced in *Escherichia coli* exists as a 'dynamic tetramer' that is rich in  $\alpha$ -helical structure<sup>127</sup>. Both studies suggested that the tetrameric helical form of  $\alpha$ -syn is resistant to aggregation and fibril formation.

The above findings have not been verified independently. Indeed, studies from our own laboratories and three other independent groups showed that  $\alpha$ -syn produced in mammalian cell lines or red blood cells or isolated from the brain tissue of mice, rats, humans or  $\alpha$ -syn transgenic animals exhibited identical mobility in native and denaturing gels to the unfolded monomeric  $\alpha$ -syn produced in *E. coli*<sup>46</sup>. NMR data of  $\alpha$ -syn in intact cells also ruled out the presence of stable or highly populated  $\alpha$ -syn oligomers and confirmed the intrinsically disordered nature of the protein in *E. coli* regardless of its purification method<sup>128</sup>. N-terminal acetylation, which was observed in native  $\alpha$ -syn isolated from cell lines and brain tissues, did not result in significant changes in the protein's structure, oligomeric state, membrane-binding properties or subcellular localization. These findings are in agreement with most studies on the oligomeric state of native  $\alpha$ -syn, which have consistently shown that  $\alpha$ -syn behaves as an unfolded monomer<sup>9,45,46</sup>. The migration of  $\alpha$ -syn with an apparent molecular weight slightly above 66 kDa in native gels and SEC is probably the result of its tendency to adopt extended conformations, and not because it exists in an oligomeric form (for example, as a tetramer), as the addition of denaturants or boiling of  $\alpha$ -syn samples from various sources did not change  $\alpha$ -syn migration<sup>128</sup>. Although Bartels *et al.*<sup>126</sup> reported that chemical crosslinking of native  $\alpha$ -syn primarily stabilized  $\alpha$ -syn tetramers, studies by several groups, including the work by Wang *et al.*<sup>127</sup>, showed that chemical or photochemical crosslinking of  $\alpha$ -syn in mammalian cells or purified  $\alpha$ -syn from *E. coli* results in the formation of a ladder of  $\alpha$ -syn species ranging from monomers to hexamers, with dimers being the major species<sup>129</sup>, consistent with the pattern expected for nonspecific crosslinking. Interestingly, SDS-resistant dimers seem to be the most commonly detected form of  $\alpha$ -syn oligomers in denaturing gels under conditions of oxidative stress<sup>49,130</sup> or in the presence of specific metals and small molecules, such as polyphenols<sup>122</sup>. These dimers are stable in the presence of dissociative conditions such as reducing agents, chemical denaturants and boiling, suggesting that they are covalently crosslinked<sup>89,122</sup>.

The development of novel tools or methodologies that allow direct detection and monitoring of changes in  $\alpha$ -syn structure and oligomeric states in living cells and/or *in vivo* is required for assessing changes in the distribution of  $\alpha$ -syn conformations and oligomeric states *in vivo* and the role of these changes in regulating  $\alpha$ -syn normal function and toxicity. This knowledge is essential for the successful design of novel strategies for the diagnosis and treatment of Parkinson's disease and related synucleinopathies.

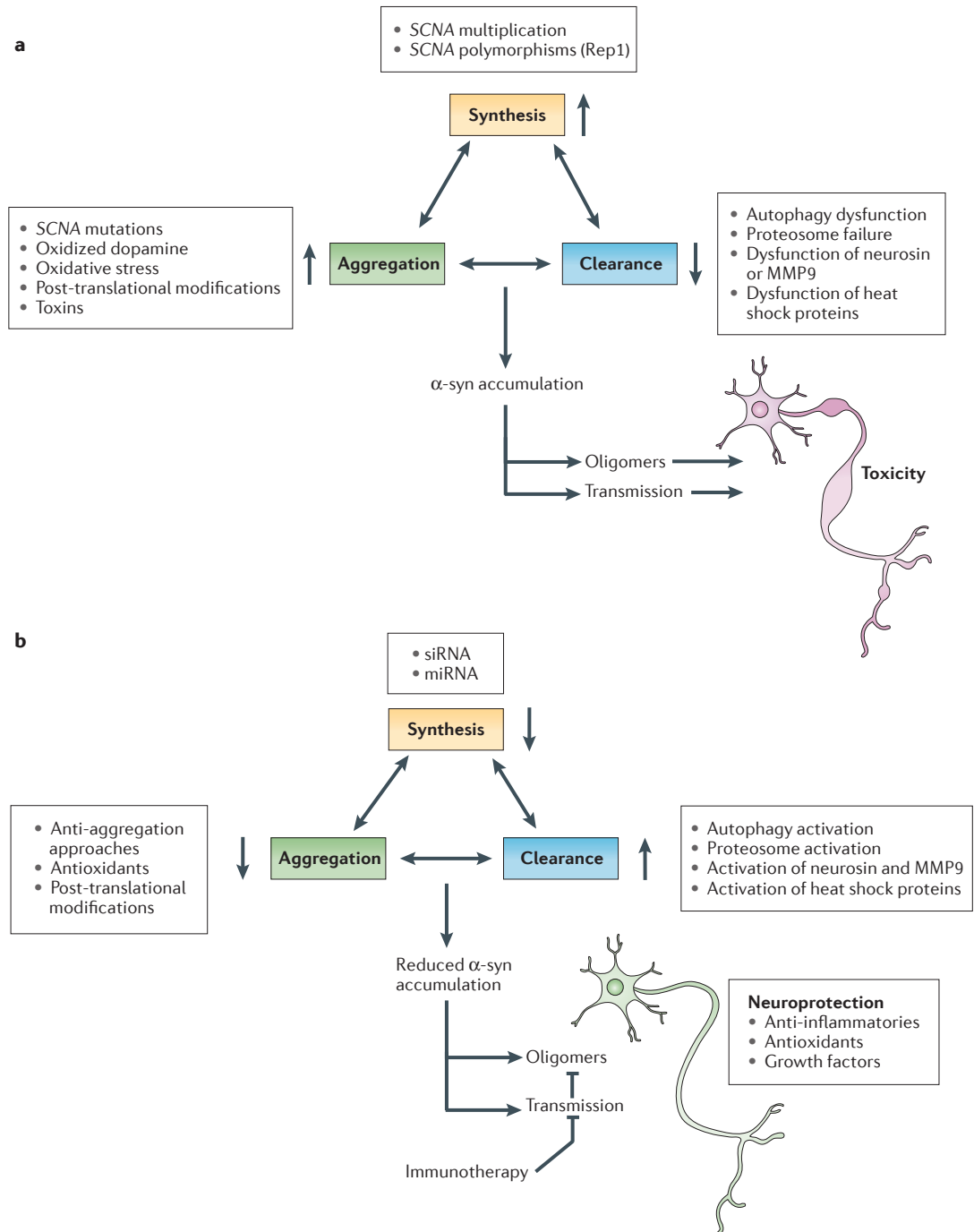
It is important to stress that in almost all of the *in vivo* studies described in the literature, the detection of oligomeric forms of  $\alpha$ -syn was solely based on indirect evidence and the use of native and/or denaturing gel electrophoresis techniques<sup>46</sup>. Thus, it remains unknown to what extent the oligomers formed *in vitro* share similar characteristics and size to those formed *in vivo* or isolated from human brains or brains of transgenic animal models of synucleinopathies. Future studies would greatly benefit from tools that allow direct monitoring of protein oligomerization in living cells.

**Toxicity of  $\alpha$ -synuclein oligomers and fibrils.** Given the diversity and heterogeneity of the oligomers that form under different conditions and induce  $\alpha$ -syn aggregation and toxicity, it is likely that various  $\alpha$ -syn oligomeric species have a role in mediating  $\alpha$ -syn toxicity in neurons and, potentially, in glial cells.

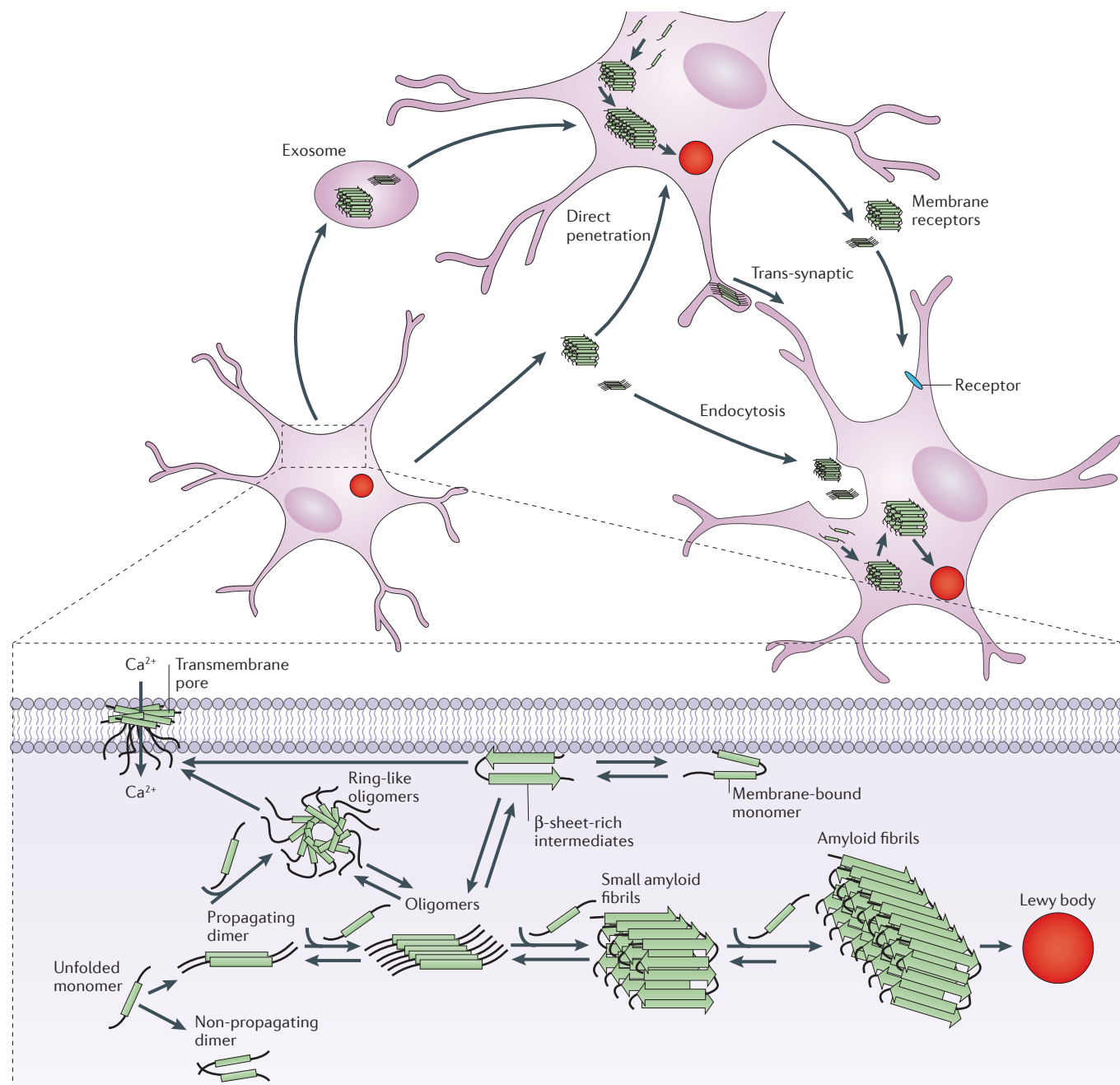
Conversion of  $\alpha$ -syn to a toxic oligomeric form (or forms) might be influenced by interactions with lipids or small molecules and post-translational modifications, including phosphorylation<sup>87</sup> of  $\alpha$ -syn at S129 and S87 (REF. 88), oxidative stress<sup>49,89</sup> and truncation<sup>11</sup>. Under *in vitro* conditions, increasing protein concentrations, long incubations at 37 °C, addition of specific metal ions (Fe<sup>2+</sup>, Cu<sup>2+</sup> and Zn<sup>2+</sup>)<sup>49,90</sup>, nitration<sup>89</sup> or application

of various ligands, such as dopamine<sup>91</sup>, can induce  $\alpha$ -syn self-assembly (FIG. 3a) and the formation of variably sized soluble aggregates that are rich in  $\beta$ -sheets. These range in stability and morphology, and some go on to form amyloid fibrils (FIG. 4).

Data collected *in vitro* and *in vivo* support the hypothesis that prefibrillar oligomers may represent the toxic species of  $\alpha$ -syn<sup>84,92</sup>. *In vitro* studies showed that annular protofibrils alter membrane permeability, resulting in an increased influx of calcium from the extracellular to intracellular space that leads to cell death<sup>86</sup>.  $\alpha$ -syn oligomers might also cause toxicity by damaging mitochondria<sup>93</sup>, triggering lysosomal leakage<sup>94</sup> or disrupting microtubules<sup>95</sup>. Moreover, a recent study showed that  $\alpha$ -syn oligomers interfere with the axonal transport of synaptic proteins such as synapsin 1, resulting in dysfunctional synapses and eventual neurodegeneration<sup>25</sup>. It is likely that  $\alpha$ -syn toxicity occurs via different mechanisms and involves different intermediates on the pathway to fibril formation. However, recent evidence suggests that the process involving the conversion of oligomers to fibrils itself contributes to  $\alpha$ -syn toxicity and the progression of neurodegeneration. Mutations that promote fibrillization (such as S129A<sup>96,97</sup>) also enhance  $\alpha$ -syn-induced toxicity and behavioural deficits in genetic rat models of PD, whereas mutations that



**Figure 3 | Cellular events controlling intracellular  $\alpha$ -synuclein levels and possible therapeutic strategies to combat  $\alpha$ -synuclein accumulation and transmission.** Intracellular  $\alpha$ -synuclein ( $\alpha$ -syn) levels are tightly regulated by the balance between the rates of  $\alpha$ -syn synthesis, clearance and aggregation. **a** | Abnormalities affecting  $\alpha$ -syn synthesis, including SNCA multiplication and polymorphisms (such as Rep1), may increase intracellular  $\alpha$ -syn levels and induce its accumulation. Accumulation may also be caused by a failure to degrade  $\alpha$ -syn. Clearance deficits might arise from failure of the ubiquitin–proteasome system, chaperone-mediated autophagy dysfunction (induced by Parkinson’s disease-linked mutations) or dysfunction of proteases (neurosin or matrix metalloproteinase 9 (MMP9)). Finally, certain SNCA mutations, post-translational modifications, oxidative stress, toxins and interaction with oxidized dopamine increase the propensity of  $\alpha$ -syn to aggregate and accumulate. **b** | Strategies to reduce  $\alpha$ -syn accumulation include decreasing  $\alpha$ -syn synthesis through use of small interfering RNA (siRNA) or microRNA (miRNA). Accumulation may also be decreased by activating mechanisms or proteins involved in clearance, such as autophagy, the proteasome, neurosin, MMP9 and heat shock proteins. Additionally, aggregation of  $\alpha$ -syn can be decreased using anti-aggregating, antioxidant or post-translational modification approaches, and immunotherapy may be used to block transmission and oligomer formation. Finally, various  $\alpha$ -syn-independent neuroprotective strategies may also be used to protect neurons from  $\alpha$ -syn-mediated toxicity.



**Figure 4 | Mechanisms of  $\alpha$ -synuclein aggregation and propagation.**  $\alpha$ -synuclein ( $\alpha$ -syn) aggregation can take place either in the cytoplasm or in association with the cellular membrane. In the cytosol, unfolded monomers interact to form initially unstable dimers, which grow slowly to generate oligomers of varying morphologies — including transient spherical and ring-like oligomers — that eventually convert to fibrils. The  $\alpha$ -syn oligomers are in equilibrium with monomers and convert to fibrils by monomer addition via a nucleated polymerization mechanism. The accumulation of these amyloid fibrils leads to the formation of intracellular inclusions called Lewy bodies. Membrane-bound monomeric  $\alpha$ -syn adopts a predominantly  $\alpha$ -helical conformation, but at high concentrations the protein undergoes a conformational change either before or coincident with its oligomerization to form membrane-bound  $\beta$ -sheet-rich structures that self-associate to form oligomers, including trans-membrane amyloid pores (the formation of which may involve several intermediates)<sup>131</sup> and fibrils. Note that the ring-like cytosolic oligomers may also associate with the membrane and form trans-membrane pores. During  $\alpha$ -syn fibrillogenesis and aggregation, the intermediate species (oligomers and amyloid fibrils) are highly toxic, affecting mitochondrial function, endoplasmic reticulum–Golgi trafficking, protein degradation and/or synaptic transmission, and these intracellular effects are thought to induce neurodegeneration. The transmembrane pores disrupt membrane integrity as well as intracellular calcium homeostasis and signalling, and may also contribute to neuronal toxicity. Interestingly,  $\alpha$ -syn oligomers and fibrils, as well as the monomers, can be transferred between cells and induce disease spreading to other brain regions. Spreading mechanisms are multiple and can occur via endocytosis, direct penetration, trans-synaptic transmission or membrane receptors. Once inside the host cells,  $\alpha$ -syn aggregates can nucleate aggregation and propagate via the mechanisms described above.

**Box 2 | Key terminology in the field of prion-like diseases**

The term 'transmission' means the conveyance of non-prion proteins, whereas the terms 'propagation', 'spreading', 'transfer' and 'dissemination' are used interchangeably to indicate the dispersal of protein over a larger area, as well as from cell to cell. 'Seeding' is synonymous with the addition of preformed aggregates to a solution of monomers. This process can eliminate the lag phase of aggregate growth that is associated with the formation of soluble assembly-competent oligomers (known as the 'nuclei') and can accelerate fibril formation. Seeding is the theoretical basis for prion infectivity, or the conveyance of prion proteins from animal to animal.

block  $\alpha$ -syn oligomerization and fibrillogenesis (such as S87E) induce significantly less  $\alpha$ -syn aggregation, fibre pathology and dopaminergic loss in rat midbrains<sup>88</sup>. Artificial mutant variants of  $\alpha$ -syn (for example, E57K  $\alpha$ -syn) that enhance the formation of  $\alpha$ -syn oligomers but not fibrils were shown to be highly toxic to dopaminergic neurons in different animal models of synucleinopathies<sup>98</sup>. In addition, Taschenberger and colleagues showed that mutations that enhance  $\alpha$ -syn oligomerization (that is, A56P and the triple mutant A30P/A56P/A76P) exhibit high toxicity, but sustained progressive loss of dopaminergic neurons was strongly dependent on the ability of the  $\alpha$ -syn variant to form fibrils *in vivo*<sup>12</sup>. These results are consistent with recent findings in the amyloid- $\beta$  field<sup>99,100</sup> and demonstrate that the process of fibril formation, rather than the fibrils themselves, has an important role in  $\alpha$ -syn toxicity and progressive neurodegeneration in PD and related disorders. Thus, it is possible that toxicity might be related to the process of aggregation rather than just to the final outcome (that is, oligomers or fibrils).

Although most of the studies mentioned here suggest that  $\alpha$ -syn oligomerization and/or fibrillogenesis has a central role in  $\alpha$ -syn toxicity, it is important to stress that most of the evidence demonstrating comparatively reduced toxicity for monomeric  $\alpha$ -syn is based on the use of recombinant proteins in extracellular toxicity assays or on the correlation between high levels of  $\alpha$ -syn oligomers (as determined by SDS-PAGE) and the progression of neurodegeneration in transgenic animals and other experimental models.

Of note, none of the available data excludes the two other possibilities, the first of which is that  $\alpha$ -syn oligomerization within specific cellular compartments may alter the distribution of functional forms of monomeric  $\alpha$ -syn or result in monomer sequestration into non-functional oligomeric forms, thus resulting in partial loss of  $\alpha$ -syn function<sup>101</sup>. The second possibility is that the native or misfolded forms of the monomeric protein may also contribute to  $\alpha$ -syn toxicity and PD pathogenesis via aggregation-independent mechanisms, including aberrant interactions with membranes, proteins and small molecules, retention in specific cellular compartments, and disruption of specific cellular processes. Unlike stable oligomeric forms of  $\alpha$ -syn, which can be easily distinguished from monomers and fibrils, the existing experimental tools do not allow the detection and characterization of different  $\alpha$ -syn monomeric conformers and thus preclude studies aimed at investigating the role of the  $\alpha$ -syn monomer in health and disease.

*Propagation and transmission of  $\alpha$ -synuclein in the pathogenesis of PD.* Under physiological conditions,  $\alpha$ -syn has been traditionally considered to be an exclusively intracellular synaptic protein that associates with vesicles<sup>15</sup>. However, recent evidence suggests that under pathological circumstances, toxic  $\alpha$ -syn oligomers could be eliminated from neurons via unusual secretory mechanisms<sup>102–104</sup> (FIG. 4). Failure of the intracellular clearance pathways, such as autophagy, might contribute to the pathological release of  $\alpha$ -syn<sup>105</sup>. Pathways leading to the release of toxic  $\alpha$ -syn oligomers include exocytosis in clear vesicles<sup>105</sup>, exosomal release<sup>103,106</sup> and penetration<sup>79,107</sup> from the donor cell membrane (FIG. 4). Interestingly, these extracellular  $\alpha$ -syn aggregates can then transfer from neuron to neuron or from neuron to glial cell<sup>105</sup>, where they can nucleate further intracellular aggregation and/or trigger neuro-inflammation to exacerbate the neurodegenerative process<sup>69,108</sup>. Supporting the potential relevance of this process in the pathogenesis of  $\alpha$ -synucleinopathies, previous studies have shown accumulation of  $\alpha$ -syn in fetal grafted neurons in patients with PD<sup>109,110</sup>, as well as in grafted neuronal precursor cells in the hippocampus<sup>69</sup> and basal ganglia<sup>111</sup> in mouse models. Interestingly,  $\alpha$ -syn has also been shown to ectopically accumulate in oligodendroglial cells in multiple system atrophy (another synucleinopathy)<sup>112</sup> and in astroglial cells in PD<sup>113,114</sup>. Moreover, the ascending distribution of the Lewy body pathology in LBD, as described by Braak<sup>113</sup>, has been interpreted to support the dissemination of  $\alpha$ -syn from subcortical to cortical brain regions. However, further *in vivo* experimental verification of this is needed. A definition for the terms of propagation, dissemination and infectivity is provided in BOX 2.

The mechanisms through which extracellular  $\alpha$ -syn oligomers transfer to other cells include endocytosis<sup>115</sup>, direct penetration<sup>116</sup>, trans-synaptic dissemination<sup>103</sup> and membrane-receptor-mediated access<sup>105</sup>. Once inside the acceptor cell,  $\alpha$ -syn oligomers could act as a focal point for further intracellular aggregation or the protein could be targeted for degradation. Although the exact process of intracellular oligomer and fibril propagation remains unknown, evidence from *in vitro* biophysical studies has consistently shown that fibrillization of  $\alpha$ -syn follows a nucleated polymerization mechanism<sup>92</sup>. This mechanism is characterized by a nucleation phase that initially involves the formation of assembly-competent oligomers (nuclei), which is followed by cooperative oligomer growth and fibril formation by monomer addition<sup>117</sup> (FIG. 4). This process can be seeded (initiated) and accelerated by the addition of preformed fibrils (the seed) and is thought to serve as the underlying mechanism for the spreading of  $\alpha$ -syn pathology in the brain. This phenomenon has been observed in a cell-based assay in which the introduction of recombinant  $\alpha$ -syn fibrils results in seeding, the recruitment of endogenous  $\alpha$ -syn and the formation of Lewy body-like inclusions<sup>44</sup>. A recent *in vivo* study has shown that inoculation of  $\alpha$ -syn transgenic mice with homogenates containing  $\alpha$ -syn protofibrils and fibrils results in considerable enhancement of the

**Nuclei**

Thermodynamically unstable oligomeric species that are capable of acting as sites for amyloid fibril growth.

**Seed**

A relatively stable entity (such as a fibril or fragmented fibril) that, when introduced into a solution containing monomeric subunits, serves as an effective nucleus and accelerates fibril formation (by eliminating the lag phase associated with nuclei formation) in a nucleation polymerization process.



$\alpha$ -syn pathology and propagation<sup>118</sup>. These observations have led to the provocative hypothesis that extracellular  $\alpha$ -syn seeds might behave in a prion-like fashion. Further experimental evidence is needed to confirm this hypothesis.

### Perspectives on therapeutic targets

Given the potential toxicity of various  $\alpha$ -syn aggregates, therapeutic approaches for synucleinopathies might involve reducing the levels or accumulation of intracellular and extracellular  $\alpha$ -syn. One way to reduce the accumulation of intracellular  $\alpha$ -syn may be to reduce the expression of this protein by silencing *SNCA* with microRNA or by repressing the *SNCA* promoter (FIG. 3b). Another approach could involve clearing  $\alpha$ -syn by activating autophagy or the proteasome, by increasing proteolytic breakdown of  $\alpha$ -syn with cathepsin D, neurosin or MMP9, or by promoting the binding of  $\alpha$ -syn to chaperone-like molecules such as  $\beta$ -syn and HSPs (FIG. 3b). A third approach would be to reduce the post-translational modifications, such as oxidation, nitration, phosphorylation and C-terminal cleavage (FIG. 3b). Two other possibilities may be to reduce aggregation or stabilize the native state of  $\alpha$ -syn (FIG. 3b).

Resolving the question of whether  $\alpha$ -syn exists as a monomer or as an oligomer under physiological and pathophysiological conditions is important not only in defining the function of this molecule but also in terms of developing therapeutic approaches for synucleinopathies. For example, if native  $\alpha$ -syn exists as a multimer, which has not yet been proven, then one therapeutic strategy for PD and DLB could involve preventing this complex from breaking apart. However, if toxic  $\alpha$ -syn oligomers are derived from single conformers, viable therapeutic approaches might include the stabilization of monomers, the stabilization of non-propagating  $\alpha$ -syn dimers, the inhibition of  $\alpha$ -syn oligomerization and the prevention of interactions between monomers and protofibrils that result in fibril growth. As *in vitro* studies of multiple amyloidogenic proteins<sup>119</sup> have shown that fibril growth and disassembly occur via monomer addition to protofibrils and disassembly, respectively,  $\alpha$ -syn toxicity would be dependent not only on the levels of oligomers but also on the concentration of the monomers. The main issue relating to developing small molecules to stabilize monomeric  $\alpha$ -syn or prevent the formation of toxic forms of  $\alpha$ -syn is that the monomer lacks a well-defined structure or hydrophobic pockets. Additionally, the identity and three-dimensional structure of toxic oligomeric species and their mode of action remain the subject of active investigation and debate.

If oligomeric  $\alpha$ -syn species are the primary cause of toxicity in synucleinopathies, then inhibition of  $\alpha$ -syn oligomerization or enhancement of  $\alpha$ -syn fibrillization should attenuate  $\alpha$ -syn toxicity and prevent neurodegeneration. Conversely, small molecules and/or mutations that stabilize toxic  $\alpha$ -syn oligomers should enhance neurodegeneration. Interestingly, the large majority of molecules that inhibit  $\alpha$ -syn fibrillization exert their effects by stabilizing high-molecular-weight oligomeric intermediates on the amyloid pathway<sup>120</sup> and protect, rather than

enhance,  $\alpha$ -syn toxicity<sup>121</sup>. This protective effect might be related to the antioxidant and anti-inflammatory properties of some of these compounds, such as polyphenols and curcuminoids<sup>122</sup>. Alternatively, it is possible that the binding of these molecules to oligomeric species of  $\alpha$ -syn blocks interactions between  $\alpha$ -syn and key mediators of  $\alpha$ -syn-associated toxicity. Interestingly, this seems to be the case for most amyloid- $\beta$  inhibitors as well<sup>123</sup>: the majority of the compounds shown to protect against amyloid- $\beta$  toxicity do not stabilize monomeric amyloid- $\beta$  and promote the accumulation of oligomers and/or non-fibrillar aggregates of various sizes and morphologies. It is possible that these molecules induce remodelling of the toxic oligomers and/or promote the formation of non-toxic aggregate or pathways. A recent study by Ayrolles-Torro *et al.*<sup>124</sup> showed that small molecule inhibitors that stabilize oligomeric forms of the prion protein attenuate prion infectivity *in vivo*, again suggesting a direct link between the processes of oligomer growth, fibrillization and prion infectivity.

The levels of toxic extracellular  $\alpha$ -syn aggregates may be reduced by targeting them with specific antibodies (passive and active immunotherapy), by increasing clearance, by blocking putative receptors for extracellular  $\alpha$ -syn in acceptor cells, by reducing  $\alpha$ -syn endocytosis and exocytosis or by targeting exosomes. Moreover, given the pro-inflammatory effects of  $\alpha$ -syn, reducing microglial or astroglial activation with non-steroidal anti-inflammatory drugs may also be a fruitful area of research<sup>125</sup>. Currently, the most viable therapeutic strategies for PD, DLB and related disorders may be to use the considerable biophysical and structural data available for the single conformation of  $\alpha$ -syn to target  $\alpha$ -syn monomers and decrease their synthesis, increase their clearance, block their interactions with oligomers or promote their stabilization. Ultimately, developing a successful therapy for synucleinopathies will require multidisciplinary research combined with multimodal therapeutics.

### Conclusions

Since the discovery of  $\alpha$ -syn in the mammalian brain, research has focused on better understanding the contributions of this natively unfolded protein to vesicular synaptic function under normal conditions. The discovery of disease-linked mutations and multiplications in *SNCA* that cause familial PD and accelerate  $\alpha$ -syn oligomerization *in vitro* has resulted in greater emphasis on elucidating the role of  $\alpha$ -syn oligomers and fibrils as well as prion-like  $\alpha$ -syn propagation in the pathogenesis of LBDs. Therapeutic interventions directed at reducing  $\alpha$ -syn synthesis, toxicity and aggregation or at increasing  $\alpha$ -syn clearance are currently being investigated in preclinical models and hold promise for developing future treatments for  $\alpha$ -synucleinopathies. We believe that more emphasis on elucidating the molecular and structural bases of the suspected physiological functions of  $\alpha$ -syn is needed. This may help us to further understand the functions of this protein and provide novel molecular targets for therapeutic intervention in  $\alpha$ -syn-linked diseases.

#### Native state

The three-dimensional structure of the protein in its normal physiological milieu in the absence of any denaturing agents or conditions.

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**Competing interests statement**

The authors declare no competing financial interests.