Opinion

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Beyond α-synuclein transfer: pathology propagation in Parkinson's disease

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α-Synuclein (α-syn) is the most abundant protein found in Lewy bodies, a hallmark of Parkinson's disease (PD), and can aggregate to form toxic oligomers and fibrillar structures. Recent studies have shown that α-syn can be transmitted between neurons and can seed the formation of toxic aggregates in recipient neurons in a prion-like manner. In addition, it is known that Lewy body pathology may spread gradually and systematically from the peripheral or enteric nervous system or olfactory bulb to specific brain regions during progression of idiopathic PD. It is therefore conceivable that α-syn species could act as seeds that drive PD progression. Here, we review recent advances from studies of α-syn cell-to-cell transfer, the current understanding of α-syn toxicity, and how these relate to progression of PD pathology.

Parkinson's disease (PD) is the second most common neurodegenerative disease in the world. The disease is primarily caused by selective loss of dopaminergic neurons in the substantia nigra par compacta (see Glossary) region of the brain. Major symptoms of PD are bradykinesia, rigidity, resting tremor and postural instability. Currently, there is no medication that can effectively stop disease progression. The primary treatment option for PD is administration of L-DOPA, which was discovered half a century ago (see Box 1 for more detailed information on PD and treatment options).

A major neuropathological hallmark in PD is the formation of Lewy neurites and Lewy bodies, protein-rich aggregates that reside in the neurons. These aggregates are highly compact and resistant to digestion, even by proteinase K, and α-syn is the major protein component.

In 1997, Polymeropoulos *et al.* showed that a single missense mutation in α-syn, Ala53Thr, leads to an inherited form of PD [1]. Since then, the *SNCA* gene (which encodes α-syn) has been linked to PD by gene duplication and triplication and at least two additional missense mutations (Ala30-Pro and Glu46Lys). This has highlighted α-syn as a protein that may play a key role in PD pathogenesis.

α-Syn has 140 amino acids and is abundantly expressed in the brain (Box 2). It is found in nearly all compartments of the neuron, but is enriched at presynaptic terminals, where it has been shown to play a role in vesicular trafficking and release by associating with the SNARE complex proteins [2,3].

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Glossary

Alzheimer's disease: the most common neurodegenerative disease worldwide. Disease onset results in debilitating memory loss, and as the disease progresses it is accompanied by other severe cognitive and functional disabilities. The disease is ultimately fatal. In most cases disease onset is seen after age 60 years, the exceptions being some genetically inherited forms. The neuropathological hallmarks of the disease are formations of extracellular β amyloid protein aggregates (plaques) and cytoplasmic hyperphosphorylated tau inclusions (pretangles, tangles).
Amyloid β (Aβ): a peptide of up to 43 amino acids in length that is cleaved from the amyloid precursor protein in neurons. Similar to α-syn, the oligomeric forms are thought to be toxic. Accumulation of Aβ is the primary pathological event in Alzheimer's disease.
Amyloidosis: conversion of often soluble proteins into well-organized large fibrillar polymers that are rich in β-sheet structures.
Bimolecular luminescence complementation system: a reporter system for measuring protein association that takes advantage of the fact that luciferase enzyme can be expressed as two separate halves that when brought together can reconstitute a functional enzyme. When each half is fused to different molecules of α-syn, as in the study cited in this review article [27], luciferase activity is regained upon α-syn dimerization.
Bovine spongiform encephalopathy: a misfolded prion-based disease that can spread from cow to human after ingestion of beef that contains misfolded prion proteins. It has a long incubation period from the time of ingestion, with at least 1 year and up to many years before disease onset.
Bradykinesia: difficulty in initiating movement and slowness of movements. A cardinal symptom of PD.
Creutzfeldt–Jacob disease: a disease caused by misfolded prion proteins in humans. Humans can inherit a genetic form or contract the disease by consuming food that contains misfolded prion proteins from other species, such as cow.
Dorsal motor nucleus of the vagal nerve: one of two parasympathetic visceromotor nuclei (dorsal motor vagal nucleus, ambiguus nucleus) in the glossopharyngeal and vagal medullary autonomic region.
Exosomes: endosome-derived 30–100-nm small membrane vesicles that are released by most cell types, including neurons.
Glossopharygeal: the glossopharyngeal nerve is the ninth of 12 pairs of cranial nerves.
Huntington's disease: a neurodegenerative disease caused by poly CAG expansion in the huntingtin gene, resulting in an extended (> 35) polyglutamine tract at the N terminus of the protein huntingtin.
Olfactory bulb: the structure in vertebrate brains that processes the sense of odors transmitted from the nasal cavity.
Proteinase K: protease with broad specificities capable of cleaving soluble proteins. The reason it does not degrade Lewy bodies is because of their compact and insoluble structure.
Rotenone: a common pesticide. It inhibits complex I of the electron transport chain.
SOD1, superoxide dismutase 1: an enzyme responsible for eliminating toxic superoxide radicals in the body. Many mutations in the gene encoding this protein have been associated with amyotrophic lateral sclerosis, a fatal neurodegenerative disease that first affects the motor neurons.
Substantia nigra: a region of the midbrain that consists of two main parts: pars reticulata and pars compacta. Cell bodies of the dopaminergic neurons that project to the striatum reside in the pars compacta.
Tau: this protein regulates microtubule polymerization in the cell. Dysregulation of the protein is associated with a range of diseases collectively termed tauopathies, of which Alzheimer's disease is the most common. In Alzheimer's disease, neurofibrillary tangles of hyperphosphorylated tau aggregate in the cytoplasm.
Vagal nerve: the tenth of 12 cranial nerve pairs.

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Box 1. Parkinson's disease

Parkinson's disease (PD) is the most common movement disorder caused by the degeneration of dopaminergic neurons in the substantia nigra pars compacta of the midbrain. Approximately 1–2% of the population in the Western world over the age of 65 years suffers from this disease.

The cardinal signatures of PD are the motor symptoms bradykinesia, rigidity, resting tremor, and postural instability. In addition, PD patients may also show other non-motor symptoms such as olfactory dysfunction, fatigue, depression, constipation and cognitive impairment.

Pathological hallmarks of PD are dopaminergic neuron death and cytoplasmic protein aggregates (Lewy bodies and Lewy neurites). α-Synuclein and ubiquitin constitute the major protein components in Lewy body pathology.

Approximately 90% of PD cases are idiopathic (non-inherited). The major risk factor for PD is aging; other known risk factors are head trauma and exposure to certain environmental toxins. There are probably many more unknown risk factors. Inherited PD has been linked to single mutation in a small number of proteins such as LRRK2, Parkin, α-syn, PINK-1 and DJ-1. The onset of PD is also likely to be caused by a combination of different mutations or by a combination of inherited mutations and other risk factors, such as aging and environmental stress.

Box 2. α-Synuclein: function and involvement in Parkinson's disease

α-Synuclein (α-syn) is a 140-amino-acid-long protein that is expressed in most neurons from the *SCNA* gene locus.

Both duplication and triplication of *SCNA* have been linked to inherited PD, as well as the missense mutations Ala30Pro, Glu46Lys and Ala53Thr. Overexpression of wild-type α-syn is sufficient to increase the risk of developing PD.

α-Syn is normally involved in vesicular trafficking by interacting with the SNARE complex proteins at the presynaptic terminals. Overexpression of the protein can disrupt the homeostasis of vesicular recycling at the terminals, leading to inhibition of neurotransmitter release.

The protein is natively unfolded, but forms α-helical structures at the N terminus on binding to lipid membranes. It can aggregate to form oligomers, ultimately leading to the formation of β-sheet-rich amyloid fibrils. Two missense mutations in α-syn that have been associated with genetically inherited PD increase the aggregation rate of α-syn, suggesting that α-syn aggregation plays a key role in its toxicity. It is widely thought that mainly the oligomeric species of α-syn are toxic to neurons. There are several different hypotheses on how α-syn oligomers mediate toxicity, including the disruption of calcium homeostasis by the formation of pores in the cell membrane, inhibition of the proteasome, and repression of the expression of pro-survival factors such as the transcription factor MEF2D.

Cleavage at its C terminus by the proteases calpain and cathepsin D, as well as phosphorylation of the protein by polo-like kinases at serine residue 129, increases its propensity to aggregate. α-Syn is the most abundant protein found in Lewy bodies, which are large protein-rich cytoplasmic aggregates found in neurons of PD patients.

α-Syn is natively unfolded but its N terminus forms α-helical structures when bound to phospholipids. It can oligomerize and this can lead to further aggregation and the formation of β-sheet-rich α-syn amyloid fibrils [4]. α-Syn can be cleaved by the proteases cathepsin D and calpain at the C terminus, which increases oligomerization propensity [5], and can be phosphorylated at Ser129 by polo-like kinases [6] (Figure 1). α-Syn is secreted from neurons and other cell types and is found in the cerebrospinal fluid (CSF) and blood in both monomeric and oligomeric forms [7–9]. However, it is unknown if secretion of α-syn has any important physiological role. Several studies have reported a difference in oligomer concentration in the CSF of PD patients relative to age-matched controls, although there is still debate as to whether α-syn is increased or decreased [8,10,11]. Two mutations in α-syn that are

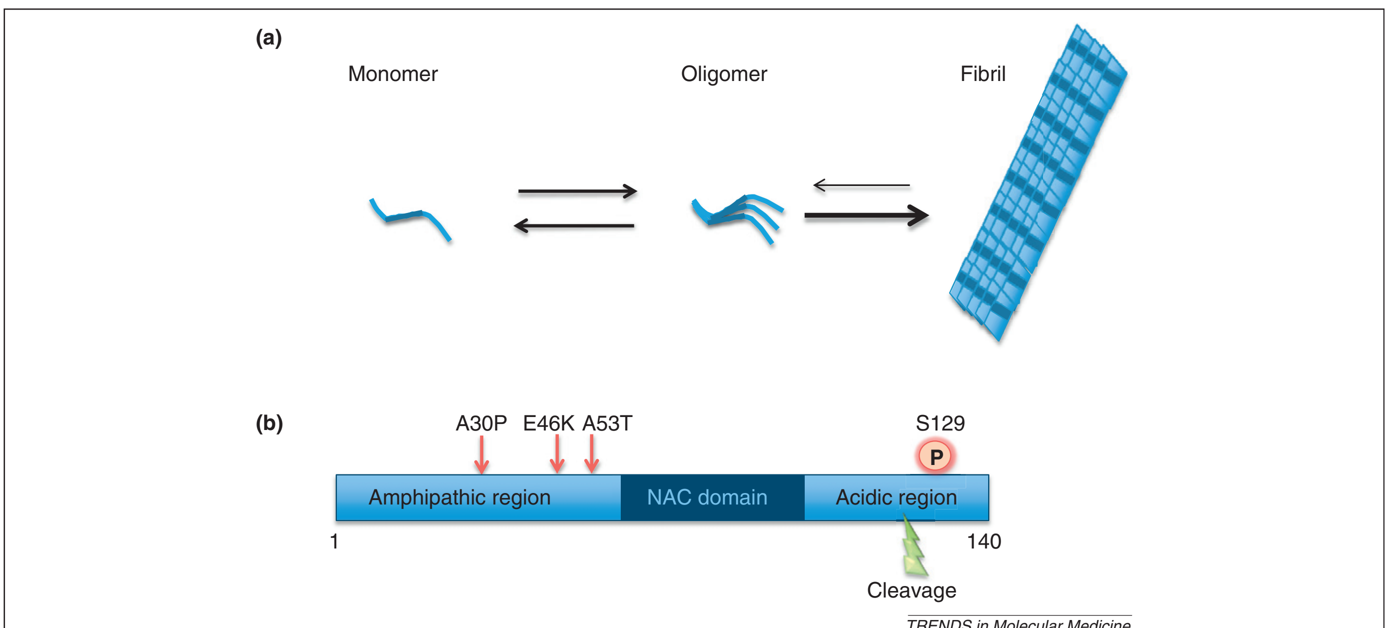

Figure 1. Aggregation and functional domains of α-syn. **(a)** Simplified scheme illustrating the progression of α-syn from its natively unfolded monomer to α-syn oligomers and then to the formation of α-syn fibrils, in which the α-syn fibrils form an amyloid β-sheet. **(b)** Illustration depicting the regions and specific sites of α-syn protein that are important for its function. Two mutations that are genetically linked to PD speed up oligomerization of α-syn, A53T and E46K, whereas a third mutation (A30P) speeds up fragmentation of the fibrils, which then accelerates the seeding process for forming new fibrils. The amphipathic N-terminal region of α-syn forms α helices when it associates with lipid membranes. This region and the hydrophobic NAC domain are particularly important for oligomerization. A fraction of α-syn is cleaved in the N terminus (between amino acids 120 and 125) by proteases such as calpain and cathepsin D, which also increases the rate of aggregation. α-Syn can be phosphorylated at Ser129 by several different polo-like and casein kinases, although α-syn is more frequently found phosphorylated in its fibril form than as a monomer.

linked to PD, Glu46Lys and Ala53Thr, dramatically increase the formation of the oligomeric and fibrillar forms of α-syn [12]. This suggests that the aggregative properties of α-syn play an important role in its toxicity. The exact mechanism of α-syn toxicity is unknown, but it could act by inhibiting protein degradation [13], disrupting calcium homeostasis [14] or causing decreased expression of the MEF2D transcription factor, which is important for neuronal survival [15].

Postmortem analysis of PD patients who received transplants of fetal mesencephalic neurons 11–22 years before death revealed Lewy bodies in the grafted neurons [16–19]. The finding of Lewy bodies in these 11- to 22-year-old transplanted neurons was unexpected, because Lewy bodies have previously only been found in much older neurons. Because Lewy bodies have never been seen in long-term grafts in Huntington's disease patients [20], Lewy body pathology in the grafts seems to be specific to PD and not caused by other events secondary to the grafting procedure itself. This observation raised the question of whether the spread of Lewy body pathology could be caused by an aggregated or misfolded form of α-syn that is secreted from the host neurons, subsequently entering grafted recipient neurons and seeding α-syn aggregation to accelerate Lewy body formation [21].

This theory was originally postulated, even before Lewy bodies were found in grafted neurons of PD patients, by Braak and colleagues, who hypothesized that PD pathology could be initiated by an unknown pathogen. Braak and coauthors mentioned misfolded α-syn as one of several candidates for such a pathogen [22]. The basis of this theory was a large body of work performed by Braak and coworkers on postmortem tissues from PD patients and control subjects. This work had shown that in idiopathic PD, which represents approximately 90% of all PD cases, Lewy body pathology spreads in a systematic manner from the peripheral or enteric nervous system or the olfactory bulb to specific brain regions during disease progression [23].

Taken together, the clinical findings of Lewy bodies in young grafted neurons and the work by Braak plausibly suggest that transfer of misfolded α-syn species between neurons could lead to α-syn aggregation in recipient neurons and spreading of Lewy body pathology. A prerequisite for this mechanism, however, would be the ability of α-syn to travel from one neuron to another, something that had not been reported at that time.

In support of this hypothesis, it was recently demonstrated that α-syn can transfer between cells both *in vitro* and *in vivo* [24,25] and that transferred α-syn can seed the aggregation of α-syn in recipient cultured neurons [25]. Since then, several other experimental studies in this now rapidly growing field have shed more light on the mechanism of α-syn cell-to-cell transfer [26–28]. Cell-to-cell transfer of several other proteins involved in major neurodegenerative disorders, including huntingtin, tau and SOD1, has also recently been described [29–31].

Collectively, transfer of these misfolded proteins between cells to seed protein aggregation in recipient cells has been termed prion-like because, as in the case of prions, a misfolded amyloid form of the proteins appears to induce spread of the pathology between cells [22,32–34]. However, a major difference between prion-like proteins and prions is that misfolded prions can induce pathology across species and individuals, as seen for bovine spongiform encephalopathy, scrapie and variant Creutzfeldt–Jacob disease [35]. This behavior has not been shown for prion-like proteins.

Systematic spreading of PD pathology in stages: The Braak hypothesis

Idiopathic (non-inherited) PD represents approximately 90% of all PD cases. Based on an extensive study of clinical human materials, Braak and coauthors concluded that aggregates (Lewy bodies) positive for α-syn appear in different parts of the brain in a systematic order at specific stages of idiopathic PD. Lewy bodies first appear in the olfactory bulb (anterior olfactory nucleus) and in the dorsal motor nucleus of the glossopharyngeal and vagal autonomic region. From the vagal nerve the gradual appearance of Lewy bodies follows an anatomical pattern in which Lewy bodies are found in the brain stem and then the midbrain, and finally spread over the cerebral cortex (Figure 2). Braak and coauthors hypothesized that the early appearance of Lewy bodies in the olfactory bulb and enteric nervous system, and the systematic spread of Lewy body pathology, might be caused by external pathogens that enter the body and trigger pathological aggregation [22,23,36–38].

The pesticide rotenone has been reported to be an environmental risk factor for the development of idiopathic PD [39]. In accordance with the theory proposed by Braak and coauthors, intragastric administration of rotenone in mice induced a systematic spread of PD pathology: Sequential appearance of α-syn inclusions in the enteric nervous system, spinal cord, brain stem and the substantia nigra was seen, which is similar to the PD pathological staging found in patients [40]. Additionally supporting a role for α-syn in the spread of PD pathology is the fact that Ala53Thr and Ala30Pro α-syn overexpression in mouse models leads to enteric nervous system abnormalities before the appearance of motor dysfunction [41].

Cell-to-cell transfer of α-syn *in vitro* and *in vivo*

In 2009, Desplats *et al*. showed that α-syn could transfer between cultured neurons and that transferred α-syn can induce death of the recipient cells [24]. Following this, we demonstrated that α-syn can not only be transferred between neurons but can also induce α-syn aggregation in recipient cells [25], suggesting that α-syn possesses prion-like properties.

To study α-syn transfer in the brain, Desplats and colleagues grafted proliferating stem cells into the hippocampus of mice overexpressing human α-syn. They found that as many as 15% of the proliferating stem cells were positive for human α-syn after only 4 weeks [24]. In a second model, the clinical situation was mimicked by grafting postmitotic fetal midbrain dopaminergic mouse neurons into the striatum of mice stably expressing human α-syn. After 6 months, a far lower percentage of grafted cells containing transferred human α-syn was observed than had been reported by Desplats and coauthors [25]. Most likely, the speed of cell-to-cell transfer depends on

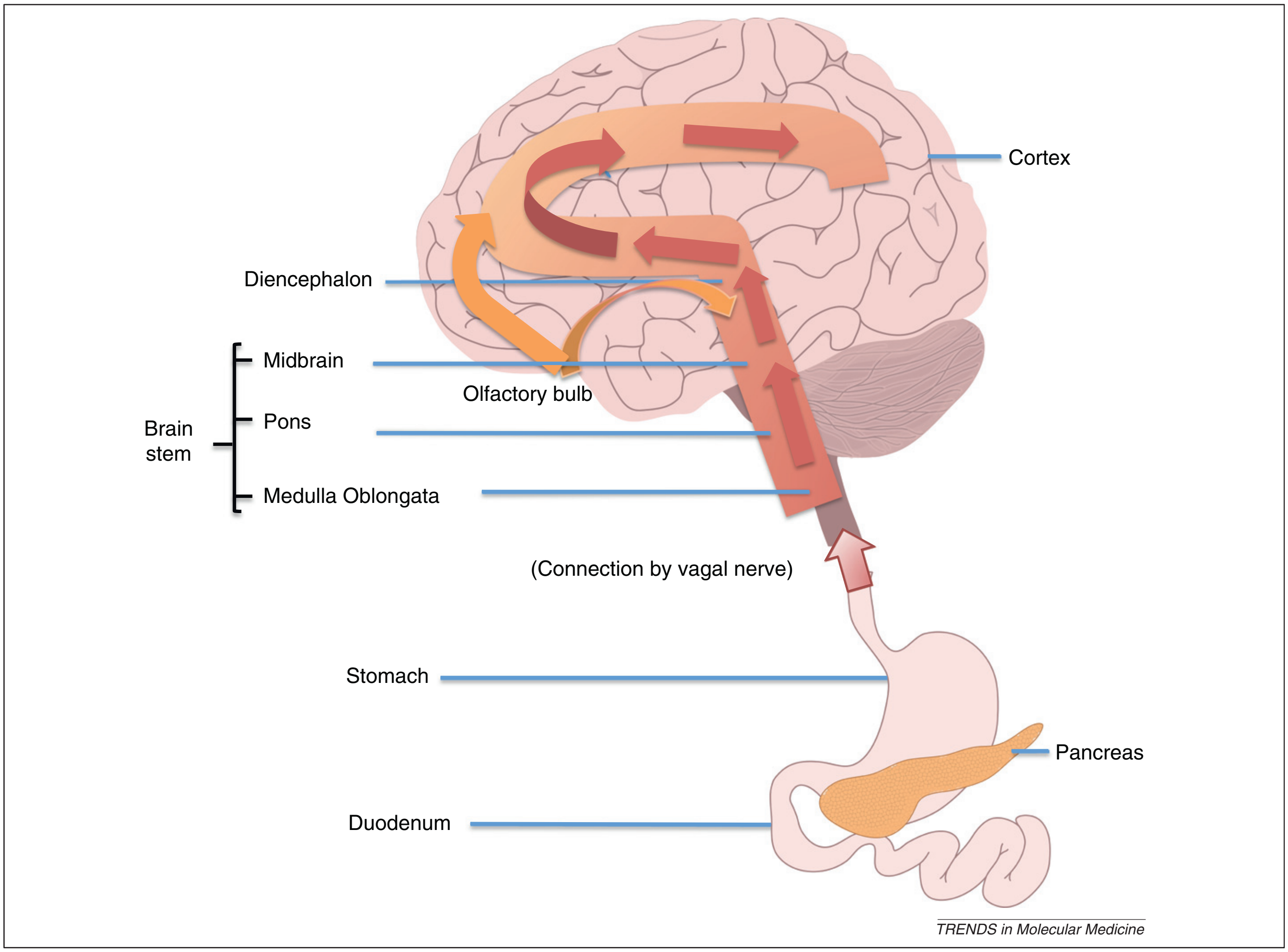

Figure 2. Spread of idiopathic PD pathology. As proposed by Braak and coauthors, Lewy body pathology may arise in the periphery/enteric nervous system, possibly in the gastrointestinal tract, and transfer to the brain stem via the glossopharyngeal and vagus nerves. Finally, it spreads to the cortex at a later stage of disease progression (red arrows). Alternatively, the pathology may initiate at the olfactory bulb and the anterior olfactory nucleus and from there spread to the midbrain and the cortex (orange arrows).

both the expression level of α-syn in the host brain and the cell type(s) used for grafting. In the clinical situation, the formation of Lewy bodies in grafts of fetal midbrain transplanted into the striata of PD patients was also a rather slow process: Lewy bodies were not detected in up to 4-year-old grafts, but were found in a subset of neurons in grafts that were 11–22 years old [16,17,19,42].

In further support of α-syn prion-like propagation *in vivo*, Mougenot *et al.* recently demonstrated that brain homogenates from mice aged 12–18 months and overexpressing Ala53Thr mutated human α-syn can trigger early onset of motor phenotypes when injected into 2-month-old mice also overexpressing Ala53Thr human α-syn. Injection of these homogenates also led to increased phosphorylation of α-syn at Ser129, an indication of increased α-syn aggregation, and to shortened lifespan in the recipient mutant mice, whereas injection of the homogenates into α-syn knockout mice had no effect on lifespan [28].

How does α-syn transfer between cells?

α-Syn can be released into the extracellular space by unconventional exocytosis or, alternatively, via exosomes [43,44], and secretion of α-syn can be increased by protein misfolding and mitochondrial stress [43]. Extracellular α-syn can subsequently be taken up by endocytosis [24,25]. Moreover, cell-to-cell transfer via tunneling nanotubes, which has been shown for prion proteins, could possibly be a mechanism for α-syn transfer [45]. The rate of cell-to-cell transfer appears to be α-syn concentration-dependent, because it is increased by inhibiting lysosomal degradation of α-syn [24,26]. Little is known about which species of α-syn are transmitted from cell to cell. However, Danzer and colleagues have shown, using a bimolecular luminescence complementation system, that oligomeric species of α-syn are transferred between neurons and that oligomeric forms of α-syn are toxic when added to cells [27]. In addition, scyllo-inositol, a cell-permeable sugar that inhibits Aβ oligomer formation, can prevent toxicity induced by exosomes containing α-syn in a cell culture model [44]. This supports the theory that α-syn oligomers travel between neurons via exosomes. (Figure 3).

It has not been explored whether neuron-to-neuron transfer of α-syn takes place across the synaptic cleft. One way to address this question *in vitro* could be the use of cell culture systems that maintain physical separation between the cell bodies and terminals of differentiated

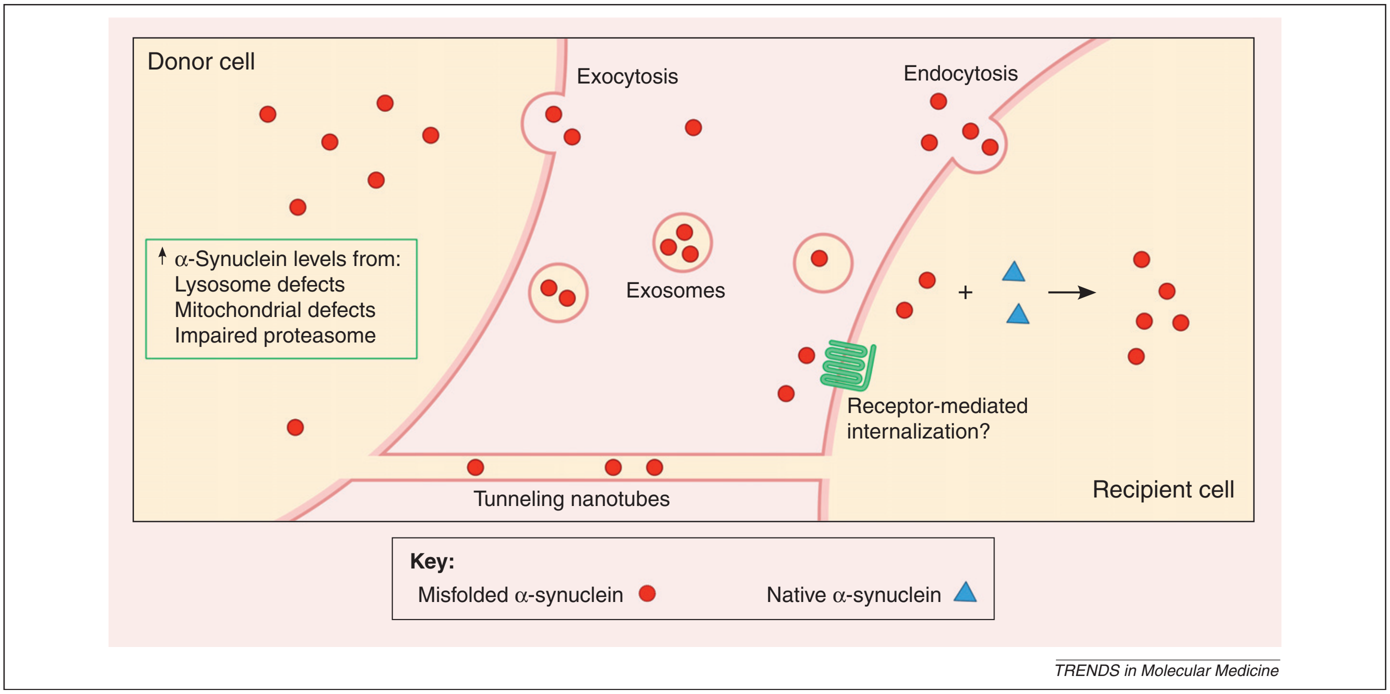

Figure 3. Possible routes for cell-to-cell transfer of α-synuclein. Exocytosis from a donor neuron followed by endocytosis by a recipient neuron is one possible route for α-syn transfer between cells. We cannot exclude that a receptor on recipient neurons could facilitate such a transfer, although there is still no experimental evidence of receptor-mediated endocytosis. Exosome-mediated transport is another route of cell-to-cell transfer of α-syn. Tunneling nanotubes, which have been shown to transport both organelles and proteins such as prions between cells, remains an unexplored possible mechanism for cell-to-cell transfer of α-syn. Misfolded α-syn (red) from the donor cell can seed the conversion of monomeric α-syn (green) into aggregated misfolded α-syn (as illustrated in the recipient cell). Impairment of proteasomal or lysosomal activity and impaired mitochondrial function are events that can lead to increases in the intracellular α-syn concentration. This concentration impacts on the rate of cell-to-cell transfer of α-syn.

neurons. Such an approach was recently used by Volpicelli-Daley *et al.* to demonstrate that aggregation of α-syn can be seeded in primary cultured neurons by preformed α-syn fibrils and this seeding of aggregation resulted in slow progression of cell death [46]. In addition, the authors demonstrated that seeding of α-syn aggregation in neurons could take place in both an anterograde and retrograde manner. Although the study did not address neuron-to-neuron transfer, it showed that α-syn seeds, which act to induce aggregation, can be taken up at the terminals and it added information on how α-syn-induced aggregation can spread inside mature neurons [46].

Toxicity of α-syn within *in vitro* and *in vivo* models

Multiple studies have also suggested that α-syn oligomers are toxic to cells [14,47]; however, when using different protocols the oligomeric species of α-syn generated *in vitro* have shown differences in terms of both structure and toxicity [48,49].

Interestingly, a few recent studies have begun to address the role of α-syn oligomers *in vivo*. Winner *et al.* showed that α-syn mutants that form oligomers, but very few fibrils, are more toxic to rats *in vivo* than wild-type and Ala53Thr α-syn [50]. Tsika *et al.* demonstrated that an oligomer 53 Å in diameter could be isolated from Ala53Thr α-syn-overexpressing mice and that this oligomer could induce cell death and seed formation of fibrillar α-syn [51]. By contrast, a recent study demonstrated that α-syn can exist as a tetramer *in vivo* that does not form fibrils [52], suggesting that not all types of oligomers can seed the formation of fibrillar α-syn. Studies using recombinant α-syn have also suggested that fibril formation is limited to some types of α-syn oligomers [48,49]. It is therefore possible that multiple forms of oligomers exist, but that only some exert a toxic effect.

Inflammation, α-syn, and PD

It is well known that neuroinflammation can play a crucial role in some models of PD [53] and that inflammation in the periphery can also potentiate neuroinflammation [54]. Viral infections might play a role in many PD cases given that the H5N1 influenza virus travels from the periphery to the central nervous system (CNS) and induces neuroinflammation, accumulation of phosphorylated α-syn and dopaminergic cell loss in a mouse model [55].

Accumulating evidence shows that α-syn also plays a role in neuroinflammation. Mouse dopaminergic neurons overexpressing wild-type or Ala53Thr human α-syn are more sensitive to neuroinflammation-induced cell death than neurons from α-syn knockout mice [56]. Lee *et al.* demonstrated that α-syn could transfer from neurons to astroglia and trigger an immune response [57]. α-Syn can also activate microglia [57,58] and increase the secretion of proinflammatory cytokines and chemokines. Finally, clearance of extracellular α-syn can rescue nigral dopaminergic neurons in a Toll-like receptor 4 expression-dependent manner [59].

In summary, it appears that α-syn could have a role in triggering and/or potentiating astroglial and microglial activation and although this could be beneficial to some extent, increased α-syn expression can also lead to increased neuroinflammation and neuronal cell death in experimental models. Further experiments are required to determine which species of α-syn trigger inflammation *in vivo* and whether this inflammation can in turn trigger the formation of toxic α-syn aggregates.

Are there parallels to other neurodegenerative diseases?

At almost the same time that cell-to-cell transfer was demonstrated for α-syn, the phenomenon was also shown in other neurodegenerative diseases for which amyloid proteins are key players [29–31].

A summary of these accumulating data is shown in Table 1, but here we highlight a few of the most important findings. Tau, involved in different tauopathies including Alzheimer's disease (AD), has been shown to transfer between cells and to seed aggregation in cell culture systems [30]. Furthermore, a prion-like role for Tau *in vivo* was demonstrated by experiments showing that injection of brain extract from mice expressing mutant Pro301Ser tau into the brains of transgenic animals expressing wild-type tau induced aggregation of wild-type tau into filaments and spread of pathology from the site of injection to neighboring brain regions [60]. A similar function for amyloid-β (Aβ), involved in AD, has also been demonstrated *in vivo*: brain extracts from humans with AD induced the formation of Aβ aggregates and associated pathology when injected into the brains of transgenic mice producing Aβ precursor protein [61]. Intriguingly, even peripherally applied Aβ was sufficient to cause accumulation of Aβ aggregates in the brains of mice [62]. These data suggest that several proteins other than prions, including α-syn [28], have prion-like properties and the ability to spread between different tissues of the body and different parts of the brain. However, prion proteins are still unique to the best of our knowledge, in the sense that unlike other amyloid proteins, they can spread across species via ingestion.

Table 1. Overview of selected publications demonstrating experimental propagation of misfolded proteins in neurodegenerative diseases

Protein	*In vitro*[a]		*In vivo*[a]		Refs
	Transfer[b]	Seeding[b]	Transfer	Seeding	
α-Syn	x		x		[24]
	x	x	x		[25]
	x	x			[27]
	x				[66]
	x				[26]
	x				[58]
		x			[14]
		x			[48]
		x			[47]
			x		[63]
	x		x		[57]
			x	x	[28]
Huntingtin	x	x			[29]
	x	x			[67]
SOD1	x	x			[31]
Tau	x	x			[30]
		x			[68]
			x	x	[60]
				x	[69]
Aβ				x	[62]
				x	[61]

[a]*In vitro* refers to studies employing cell culture models, whereas *in vivo* refers to studies using animal models.

[b]Cell-to-cell transfer (transfer) and/or seeding of protein aggregation for the formation of amyloid fibrils (seeding).

Concluding remarks and future perspectives

α-Syn cell-to-cell transfer is now well established in cell culture models and has been demonstrated in animal models [24,25]. It has also been established that α-syn possesses prion-like properties *in vivo* [28]. Taken together, these data support the idea that α-syn propagation has an important role in the spread of PD pathology during disease progression. However, it is still not known whether formation of toxic α-syn aggregates in the olfactory bulb or the enteric nervous system is sufficient to induce PD. Therefore, experiments are needed to address this issue. Development of drugs that inhibit cell-to-cell transfer of α-syn would also help to elucidate the importance of α-syn transfer in animal models, apart from their obvious potential use in treating PD.

A treatment option for PD that could be affected by the newly discovered prion-like property of α-syn is grafting of dopaminergic neurons into the PD brain. Clinical studies from several laboratories have shown Lewy body pathology in grafted neurons when human fetal midbrain neurons were grafted into the striata of PD patients 9–16 years before death. However, only a small proportion of grafted neurons exhibited Lewy bodies, and it appears that most of the neurons could still provide long-term beneficial effects for the patients [16–18]. In contrast to the comparatively higher proportion of Lewy bodies found in other studies [16,18,19,63], Isacson and coworkers did not observe Lewy body formation in transplants after similar postoperative periods [64], although they did describe the appearance of a low number of Lewy bodies in a younger subject in their follow-up report [65]. Interestingly, these discrepancies may be related to the different techniques used in the transplantations, such as tissue preparations, and to the different degree of inflammatory responses to the grafts [65]. Thus, seeding of α-syn aggregation in grafted cells, caused by cell-to-cell transfer from PD brain host neurons does not necessarily exclude graft-based therapy with fetal midbrain derived or stem-cell-derived neurons, but it does provide an additional concern for this type of therapy.

We now know that the aggregative properties of α-syn play a role in its toxicity, but there is no general consensus regarding which α-syn species are toxic *in vivo* and whether some forms of oligomers, for example, the newly discovered tetramer [52], are physiologically functional. Because α-syn gene knockout has few or no consequences in mouse models, compounds that cause degradation of α-syn protein or mRNA or inhibit α-syn oligomer formation are potential drug candidates for future anti-PD therapies.

Box 3. Pending questions for the role of α-syn in PD

- Which species of oligomers are toxic *in vivo*?
- Which species of α-syn can transfer between cells?
- What will the effect of drugs that can block α-syn aggregation and cell-to cell transfer be? Would both types of drug inhibit PD progression?
- Does the prion-like behavior of α-syn play a crucial role for PD disease progression?

In summary, we believe that the prion-like property of α-syn plays an important role in the progression and perhaps even the initiation of PD pathology. However, despite accumulating evidence, we cannot yet say for certain that this is the case (Box 3). If the prion-like property of α-syn is important for progression of PD pathology, then we anticipate that this holds promise as a new avenue for the development of future PD therapeutics. This may also be true for other neurodegenerative diseases in which proteins with a prion-like property are key players.

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