# What's to like about the prion-like hypothesis for the spreading of aggregated α-synuclein in Parkinson disease?

Christopher J.R. Dunning,[1,]* Sonia George[1] and Patrik Brundin[1,2]

[1]Neuronal Survival Unit; Wallenberg Neuroscience Center; Lund University; Lund, Sweden; [2]Van Andel Research Institute; Center for Neurodegenerative Science; Grand Rapids, MI USA

α-Synuclein is a key protein in Parkinson disease. Not only is it the major protein component of Lewy bodies, but it is implicated in several cellular processes that are disrupted in Parkinson disease. Misfolded α-synuclein has also been shown to spread from cell-to-cell and, in a prion-like fashion, trigger aggregation of α-synuclein in the recipient cell. In this mini-review we explore the evidence that misfolded α-synuclein underlies the spread of pathology in Parkinson disease and discuss why it should be considered a prion-like protein.

## Introduction

Parkinson disease (PD) is the second most prevalent neurodegenerative disease, and the most common synucleinopathy. Synucleinopathies feature aggregated α-synuclein (α-syn) in intracellular inclusion bodies, which are termed Lewy bodies (LB) or Lewy neurites (LN) depending on their location. They are the classical neuropathological hallmark of PD and were first described by Friedrich Lewy a century ago.[1] It is not clear why LB and LN form, or what impact these inclusions have on cell function. Proteomic analysis reveals they are comprised of greater than 100 different proteins,[2] the major protein being α-syn. Since the discoveries that α-syn was the major protein component of Lewy aggregates,[3] and that point mutations and genetic variation in the α-syn gene can cause rare forms of dominantly inherited PD, it has been a major focus for PD researchers. More recently, research on this little understood protein has taken an additional direction with the discovery that not only is α-syn the major protein component of LB and LN, but that intercellular exchange of the misfolded form might actually play a role in spreading α-syn pathology from cell-to-cell.

α-Syn is a 140 amino acid protein of predominantly presynaptic localization in neurons, although it is ubiquitously expressed.[2,4] The protein is comprised of 3 domains: (1) an N-terminal lipid binding α-helix, (2) a non-amyloidβ component (NAC) domain and (3) an unstructured C-terminus. All three regions are important for the misfolding of α-syn, a process critical for the induction of synucleinopathies. α-Syn is primarily a natively unfolded cytosolic protein, however via its N-terminal α-helix, it does bind to membranes, upon which it adopts an α-helical structure.[5] It is also on the membrane that α-syn can misfold and begin to form aggregates.[6] When misfolding occurs, the random coil of the NAC region forms β-sheets, leading to protofibril and fibril formation.[7] The C-terminus plays a role in inhibiting this fibril formation, but is also home to several phosphorylation sites, of which hyperphosphorylation at S129 (pS129) is associated with α-syn pathology.[8]

## α-Syn and Neurodegeneration

The link between α-syn and PD is strong with three missense mutations in the α-syn gene (PARK1/SNCA) causing autosomal dominant PD.[9-11] Multiplications of SNCA[12,13] lead to parkinsonian symptoms and genetic variations in the non-coding regions of the gene also increase an individual's susceptibility to PD.[14] α-Syn levels also increase with age,[15,16] which correlates with the increased incidence of PD in the aged.[17]

The direct link between α-syn pathology and PD pathology, including death of dopaminergic neurons, is not entirely clear, with some even suggesting α-syn pathology in the form of LB and LN is neuroprotective.[18,19] Despite this, several studies have shown that misfolded α-syn has multiple detrimental effects on a number of cellular processes that could lead to neurodegeneration. Disruptions to these processes are also associated with normal aging, and also impact on the function and homeostasis of α-syn. This raises the question which is the chicken and which is the egg? Do cellular dysfunctions that have been associated with normal aging, e.g., oxidative stress, result in corruption of α-syn? Alternatively, higher cytoplasmic levels of α-syn associated with normal aging might increase the likelihood that other unknown, stochastic events that trigger α-syn misfolding take place. It is also possible that clearance of small quantities of misfolded proteins, e.g., α-syn, is impaired in aged cells leading to the seeding or large aggregates. Whatever the answer, it is clear there is a dynamic interplay between α-syn and many cellular processes and that this protein is likely to play a crucial role in PD pathogenesis.

*Correspondence to: Christopher J.R. Dunning;
Email: Christopher.Dunning@med.lu.se

Perhaps the concept of age-related cellular dysfunction is most important when discussing why α-syn pathology does not spread equally well to all brain regions, and it could explain why some cells are affected by α-syn pathology whereas neighboring cells are sometimes completely unaffected. Thus, cells already challenged by i.e., already high levels of oxidative stress, which has been suggested to apply to substantia nigra dopaminergic neurons, are likely to be more susceptible to a seeding mechanism following uptake of misfolded α-syn.

**Oxidative stress.** Oxidative stress is prevalent in the parkinsonian brain. This in turn results in damage to lipids, proteins and DNA (especially mitochondrial DNA).[20] Dopaminergic neurons in particular are vulnerable to oxidative stress as dopamine itself can undergo oxidation, thus generating reactive oxygen species (ROS).[21] Dopamine is usually sequestered into synaptic vesicles soon after synthesis where it is protected from oxidation.[21] One suggested function of α-syn is in the regulation of vesicular uptake and turnover of dopamine at the synapse,[22] and overexpression of α-syn has been shown to cause synaptic dysfunction leading to alterations in neurotransmitter release.[23,24] With uptake of the neurotransmitter perturbed due to non-functional α-syn, cytosolic, and thus oxidation prone, dopamine accumulates.[25] Dopamine metabolites have also been suggested to promote α-syn aggregation[26] and dopamine-α-syn adducts can stabilize potentially toxic α-syn protofibrils, thus preventing the conversion of protofibrils to fibrils.[27]

The relationship between α-syn misfolding and oxidative stress is likely to be a self-perpetuating one; α-syn aggregates in an environment rich in ROS, which in turn promotes further ROS production. Thus, evidence suggests that α-syn aggregation promotes generation of ROS.[28] Specifically, oligomeric species of α-syn induce higher levels of ROS production than monomeric and fibrillar species.[29] Further, α-syn preferentially aggregates in the presence of both cytochrome C and hydrogen peroxide (both sources of oxidative stress) to form dimers and insoluble aggregates which are likely precursors of LB.[30] In line with this, LBs contain modified α-syn in the form of nitrated and oxidized species.[31]

**Mitochondrial dysfunction.** The notion that PD and mitochondrial dysfunction are connected has been around for over two decades.[20] PD patients exhibit decreased activity of complex I of the electron transport chain[32] and mitochondrial DNA polymorphisms and haplotypes modulate risk for the disease.[33,34] In addition, several genes encoding mitochondrial proteins are linked to inherited forms of PD.[35]

In this context, it is particularly interesting that α-syn interacts directly with mitochondria and may indeed contain a mitochondrial targeting signal.[36] Two possible functions have been attributed to mitochondrial α-syn, i.e., regulation of mitochondrial dynamics[37] and maintenance of mitochondrial calcium homeostasis.[38] Overexpression of α-syn leads to mitochondrial dysfunction by interfering with complex I[39] and fragmenting the mitochondrial network.[40] Misfolded α-syn also accumulates within both mitochondrial membranes,[36] leading to disruption of ATP synthesis and disruption of the mitochondrial membrane potential.[41] Mitochondrial dysfunction has also been shown to destabilize the microtubule network, in turn leading to α-syn oligomerization.[42]

**Neuroinflammation.** The role of microglia and astrocytes, the resident immune cells of the brain, in PD is not clear. Both are activated not just in PD but also in several other neurodegenerative diseases. The activation of these cells, as part of a neuroinflammatory response, obviously implicates the immune system in PD but it does not clarify whether neuroinflammation is a primary event or a secondary consequence in PD pathogenesis (for review, see ref. 43). In some genetic forms of PD, a primary role of the immune system seems likely. Thus, mutations in two immune-related genes DJ-1[44] and LRRK2[45] cause inherited forms of PD. In other forms of PD α-syn might promote an immune response in at least one of two ways. Either α-syn secreted into the extracellular space could stimulate microglia directly,[4] or by causing neuronal death, misfolded α-syn might indirectly cause microglial activation.[46]

Overexpression of α-syn, both the normal and mutant forms, stimulates microglia, resulting in the release of pro-inflammatory cytokines, nitric acid, complements and ROS, promoting further inflammation and neurodegeneration.[47,48] In transgenic mice, microglia activation is related spatially and temporally to α-syn overexpression.[49] Post-translational modifications of α-syn are also associated with activated microglia.[50] While the nuances of the interrelationship between α-syn and neuroinflammation are yet to be clarified, it is clear that the two are dynamically linked.

**Autophagy.** Under control conditions, α-syn in its native form is degraded by chaperone mediated autophagy (CMA).[51] However mutant forms, including post-translationally modified α-syn, block this process, leading to an increase in cytosolic α-syn inclusions.[52] Mutant α-syn also blocks receptors of CMA, thus preventing other CMA targets from binding.[53] When α-syn is overexpressed, the expression of the CMA receptor protein LAMP-2A is also increased, however if the increased levels of α-syn result in the formation of oligomers, then CMA is unable to degrade these.[51,54] α-Syn pathology is associated with an upregulation in autophagic activity in transgenic mice expressing mutant α-syn.[55] With CMA blocked, α-syn can be degraded though macroautophagy (commonly referred to as autophagy). This pathway also has the potential to be detrimental to the cell resulting in autophagic cell death.[52] Overexpression of α-syn, however, can also impair autophagy;[56] although some studies indicate that enhancing autophagy can have a protective effect.[57]

α-Syn and autophagy can also be linked to mitochondrial dysfunction. Two genes essential to mitophagy (autophagy of mitochondria), PARKIN and PINK1, are linked to PD. Overexpression of α-syn, in particular A53T mutant, results in an increase in mitophagy. In these cells there is a drastic reduction in the number and size of mitochondria, a process for which PARKIN has been found to be essential.[58]

## The Prion-Like Hypothesis

While Braak and colleagues proposed that Lewy pathology spreads throughout the brain and this spread correlates with the disease stage,[59] they were uncertain what the spreading agent

might be and speculated that it, for example, is a virus. It was not until autopsies were performed on a small number of PD patients who had undergone neural transplantation more than a decade before death that the prion-like hypothesis gained momentum. These particular patients had received embryonic neuronal cell transplants in the striatum. At autopsy it was discovered that the young transplanted neurons were positive for cytosolic α-syn and contained LB,[60,61] a surprise given the young age of the neurons and that there was no reason to believe that the graft tissue donors were afflicted by PD.

The initial observation of α-syn pathology in the grafted neurons sparked a discussion of several possible explanations.[62] Oxidative stress, inflammation and excitotoxicity all present in the parkinsonian brain can, to varying degrees, explain the increase in α-syn levels and the presence of α-syn pathology.[62] It also led to speculation that the α-syn present originated in neighboring brain areas which were positive for LB pathology.[60] Possibly, all the factors mentioned above are responsible for the appearance of α-syn pathology in the graft, with the aged brain environment responsible for the increased cytosolic α-syn but the transfer of oligomeric α-syn seeds responsible for the formation of LB.[63]

Subsequent studies looking at cell-to-cell transfer have shown that α-syn is capable of transfer between cells and some of the mechanisms behind this spread have been elucidated (see review in ref. 64). Of course, for a protein to qualify as prion-like, it must also corrupt the native form of endogenous protein in the recipient cell. In the case of α-syn, this corruption is believed to occur with the transferred (misfolded) protein acting as seed, initiating the aggregation of the endogenous protein.

**Transfer.** Braak and colleagues proposed that the initial site of α-syn pathology is either the olfactory bulb or the enteric nervous system, or that both are involved early on.[65] While numerous autopsies lend weight to this hypothesis, modeling the spread under experimental conditions is only just starting to yield results. In experiments analogous to the human transplants described above, host derived α-syn has been identified in grafted neurons in the brain of rodents.[66,67] In the case of the experiment of Angot et al.,[66] the transferred protein recruited α-syn in the recipient cell. Similar experiments have also been performed using aged brains from PD symptomatic mice, with pathology spreading from the site of injection throughout the brain.[68,69] Spread of pathology through the enteric system has also been observed in A53T transgenic mice.[70] Human brain tissue extracts from a dementia with Lewy body patient were injected into the gastric wall of these mice and over the course of 4 months, α-syn aggregates accumulated in the myenteric neurons.

Perhaps the best indication that α-syn pathology does spread will come from studies using human tissue. While the initial experiments that led to this hypothesis came from autopsy, the nature of this technique means longitudinal studies are impossible. With the suggestion that the gut may be a site where α-syn aggregation is initially triggered, it means that examining gut biopsies from people who later develop PD may be a viable tool to address this hypothesis. Indeed, α-syn aggregates were identified in colonic biopsies from PD patients that had undergone colonic

biopsy 2–5 years before the onset of PD symptoms, by contrast, α-syn aggregates were absent in biopsies from control individuals who did not develop PD.[71] Taken together these intriguing findings suggest that α-syn pathology might start in the enteric nervous system before it spreads to the central nervous system and the substantia nigra.

The focus on intercellular protein transfer has recently been on prion-like proteins, such as α-syn, amyloid-β (Aβ), Tau, SOD-1 and prion. However, it is worthwhile to remember that other proteins that are not associated with disease can also undergo intercellular transport. Proteins can be exchanged between neighboring neurons at the synapse,[72] and for many years it has been known that, e.g., proteins used to trace neuronal pathways can be transported transsynaptically.[73] The precise mechanism of transport is not known, and several possible explanations have been entertained. For example, exosomes can carry biological material, be it protein, mRNA or miRNA, over large distances. This is believed to be important for cellular communication, particularly by the immune system, although most cells studied to date secrete exosomes. Presently, somewhere in the order of 11,000 proteins have been identified in exosomes.[74] Another example of a possible mode of intercellular transport of proteins are tunneling nanotubes, which are able to exchange whole organelles and cytosolic proteins between cells.[75]

**Seeding.** A number of groups have observed spreading of α-syn,[64] but observing any subsequent seeding has proved more of a challenge. The reason for this could be in the number of factors required for this seeding event to take place, with these processes difficult to model experimentally. Despite this, a number of instances have appeared in the literature recently. While some groups have seen evidence to suggest seeding is occurring, it was the use of pre-formed fibrils in vitro where the seeding effect of α-syn was perhaps best demonstrated.[76,77] Not only did incubation of neurons with the pre-formed fibrils lead to aggregation of endogenous α-syn, but it had drastic consequences to the cell with wide spread synaptic dysfunction.[77] The same group showed similar results in vivo where the pre-formed fibrils were injected into the striatum and cortex of asymptomatic α-syn transgenic mice.[68] Ninety days post injection, α-syn pathology had spread throughout the brain, while mice injected with vehicle remained free of α-syn pathology, indicating that the presence of α-syn fibrils is enough to trigger and spread α-syn pathology. Perhaps even more significantly, when pre-formed fibrils were injected into wildtype mice, aggregated α-syn was again observed as shown previously for the transgenic mice. The key difference being in these mice that by 180 d post-injection, significant PD pathology, including dopaminergic neurodegeneration was observed correlating with the appearance of aggregated α-syn.[78]

While the experiments performed with pre-formed fibrils were conclusive, it has been debated why it is necessary to use recombinant protein to induce these effects. The debate is similar to the Aβ and the Alzheimer field, where the outcome is highly dependent on the specific protocols used to aggregate the protein prior to intracerebral injection.[79] The advantage of using recombinant protein is that the seeding process can be controlled carefully in the test tube before the intracerebral injection is made,

which does not apply to a crude mix such as a brain homogenate. However, as long as recombinant protein is used to initiate seeding and spread of pathology debate will remain, therefore future experiments need to address if similar results are obtained using seeds originating from a cell and not a test tube.

## Why a Prion-Like Hypothesis Makes Sense

Why is α-syn described as prion-like and not prion? Certainly, as has been outlined here, misfolded α-syn could be responsible for the cellular transmission of PD pathology, although the underlying mechanisms remain elusive. On the other hand, the pathobiology of prion itself is relatively poorly understood. It is not known how it causes damage to its host cell, what form of prion oligomers are toxic, nor is its native function known. What we do know is that it is capable of cell-to-cell transmission and that it is a change in conformation from the normal cellular form (PrP$^C$) to the toxic form (PrP$^{Sc}$) that causes toxicity. It is assumed that PrP$^{Sc}$ acts as a seed for PrP$^C$ aggregation, a process that has been described as akin to that of crystal formation.[80] While a seeding or nucleation process has also been described for α-syn, it has yet to be studied in the same level of detail as PrP$^{Sc}$ and further work is needed to clarify whether nucleation does indeed occur. Perhaps the most striking difference between PrP$^{Sc}$ and α-syn however is in its transmission. While biologically it seems that α-syn in its toxic amyloid form is transmissible from one cell to another,[68,70] PrP$^{Sc}$ is the only protein that has been shown to be transmissible at the organism level, i.e., from one individual to another. There is currently no evidence that misfolded α-syn can be transmitted from one individual to another. This lack of transmissibility might be coupled to the fact that α-syn has not been shown to be capable of self-replication. Thus with current knowledge, it is clear that α-syn is not a prion protein.

What the experiments highlighted above suggest though is that α-syn can act as a prion-like protein and the dynamic interplay that exists between misfolded α-syn and cellular dysfunction. Of course this interplay on its own could explain neurodegeneration in the PD brain.[4] However, it does not fully explain the slow degeneration associated with the disease. There is little debate that PD is a disease that spreads. Pre-motor symptoms can appear decades before the on-set of motor-symptoms,[81] and while not every PD patient exactly fits the staging scheme described by Braak,[82] the degeneration observed in the majority of cases suggests a slow, progressive spread of neuropathology. The question remains, what causes this spread? And why are some neurons more vulnerable than others?

We know that transferred α-syn can recruit host α-syn, potentially depleting it from its site of normal function e.g., the synapse, which in turn can lead to a reduction in synaptic function, the consequences of which were discussed briefly above. If the transferred protein is misfolded, and consequently causes more protein to misfold, then it is possible α-syn acquires a "novel"

function, that of a protein complex that binds and disrupts membranes, again causing stress to the cell. We also know that α-syn levels increase with age. As with all proteins, sometimes it misfolds. As our cells contain more and more α-syn, the proportion of misfolded α-syn will increase, causing greater burden to the cellular machinery tasked with dealing with misfolded protein.

While LBs or LNs can appear in most, if not all, cells of the brain, certain cells appear more vulnerable to degeneration than others.[83] The reason for this is likely to lie in the cells ability to handle toxic protein i.e., misfolded α-syn. A cell with a high energy requirement will be less able to cope with an influx of toxic protein, likewise a cell which is rich in ROS is likely to provide the right environment for further α-syn aggregation and subsequent spread of pathology. The spread of pathology is also a very slow process, suggesting that the cell can cope for a certain period of time and only when a threshold is reached does sufficient degeneration occur.

## Is α-Synuclein a Prion-Like Protein?

There are three factors that have been described for α-syn that are common to other prion-like proteins. (1) α-Syn has different conformations. Under physiological conditions it is predominantly unstructured or α-helical (possibly organized in a tetramer) in structure. The pathological form of α-syn consists of oligomers and fibrils rich in β-sheets. (2) α-Syn transfers from cell-to-cell. Whether or not it transfers to a higher degree than other proteins is unknown, but it is clear that aggregated α-syn can transfer between cells. (3) Aggregated α-syn has been shown to propagate in vivo. Direct catalysis of misfolding of endogenous α-syn has not been observed in these instances, but a time dependent increase in aggregation is clear.

The trigger that leads to misfolding and aggregation of α-syn remains unknown. In a number of rare cases, mutations in the α-syn gene alone may be enough, but in the majority of cases a number of factors are likely to contribute. What is becoming clear however, whatever the trigger, misfolded α-syn is the likely bullet, spreading synuclein pathology throughout the brain.

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