Protein trapping leads to altered synaptic proteostasis in synucleinopathies

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Parkinson’s disease (PD) is associated with the accumulation of alpha-synuclein (aSyn) in intracellular inclusions known as Lewy bodies and Lewy neurites. Under physiological conditions, aSyn is found at the presynaptic terminal and exists in a dynamic equilibrium between soluble, membrane-associated and aggregated forms. Emerging evidence suggests that, under pathological conditions, aSyn begins to accumulate and acquire a toxic function at the synapse, impairing their normal function and connectivity. However, the precise molecular mechanisms linking aSyn accumulation and synaptic dysfunction are still elusive. Here, we provide an overview of our current findings and discuss the hypothesis that certain aSyn aggregates may interact with proteins with whom aSyn normally does not interact with, thereby trapping them and preventing them from performing their normal functions in the cell. We posit that such abnormal interactions start to occur during the prodromal stages of PD, eventually resulting in the overt manifestation of clinical features. Therefore, understanding the nature and behaviour of toxic aSyn species and their contribution to aSyn-mediated toxicity is crucial for the development of therapeutic strategies capable of modifying disease progression in PD and other synucleinopathies.

Introduction

Parkinson’s disease (PD) and other synucleinopathies are neurodegenerative diseases characterized by the presence of intraneuronal inclusions known as Lewy bodies (LBs) and Lewy neurites (LNs). These inclusions are composed primarily of alpha-synuclein (aSyn), but they also contain neurofilaments, ubiquitin, synphilin-1, torsinA and heat shock proteins, among other components [1–3]. aSyn is a small protein of 140 amino acids that belongs to the synuclein family. It is abundant in neurons of the mammalian brain, where it localizes predominantly in presynaptic terminals [4–6]. The precise function of aSyn is still unknown but it is known to interact with various proteins, like actin [7,8], tubulin, synphilin-1, microtubule-associated protein tau, proteins, preformed fibrils; ROS, reactive oxygen species; SOD2, superoxide dismutase 2; SRC, spare respiratory capacity.

Abbreviations
aSyn, alpha-synuclein; DBL, dementia with Lewy body; ER, endoplasmic reticulum; HSP10, 10 kDa heat shock protein; HSP27, 27 kDa heat shock protein; HSP60, 60 kDa heat shock protein; HSP70, 70 kDa heat shock protein; HSP90, 90 kDa heat shock protein; HtrA2, high temperature-regulated A2; LBs, Lewy bodies; LNs, Lewy neurites; paSyn, S129-phosphorylated aSyn; PD, Parkinson’s disease; PFFs, preformed fibrils; ROS, reactive oxygen species; SOD2, superoxide dismutase 2; SRC, spare respiratory capacity.
tyrosine hydroxylase, protein kinase c or Bel-2-associated death protein [9]. aSyn is involved in the maintenance of cell structure and protein trafficking via its interactions with diverse cytoskeletal proteins [10–13]. It is also involved in transmembrane transport and in the formation of synaptic vesicles [14–16], in stabilizing the effects of SNARE-complex assembly [17,18] and in the modulation of synaptic functions [19–22]. As an intrinsically disordered protein, that can adopt different conformations, aSyn may also behave like a hub within protein interaction networks, interacting and influencing, directly or indirectly, other partners.

When overexpressed in model systems, aSyn can aggregate and affect mitochondrial calcium homeostasis [23], and protein import [24] and turnover [25]. In some models, overexpression promotes cytotoxicity, impairs autophagy and mitochondrial processes due to the generation of reactive oxygen species (ROS), increases sensitivity to oxidative stress, compromises vesicular transport and disrupts the trafficking between the endoplasmic reticulum (ER) and Golgi [26–32].

Microtubules are important structures for the intracellular transport of molecules in the cytosol, and also for the integrity of cell shape. In rat, primary neurons infected with viruses encoding for aSyn [33] exhibit neurite degeneration and membrane blebbing, consistent with detrimental effects of aSyn on tubulin polymerization [34].

aSyn can also mediate the cytoplasmic retention of DNA methyltransferase 1 (Dnmt1), resulting in DNA hypomethylation and up-regulation of SNCA and other PD-related genes. In human postmortem brains of PD and/or dementia with Lewy body (DLB) patients, the reduction of nuclear levels of Dnmt1 and DNA methylation [35], the accumulation of Elk-1 (phosphorylated transcription factor) within aSyn glial cytoplasmic inclusions [36–38] and cytoplasmic aggregates of phospho-CREB [39,40] were also reported.

All of these cellular pathological events are likely to contribute to the onset and development of neurodegeneration. Nevertheless, the precise trigger(s) of pathogenesis in PD and other synucleinopathies remain unclear.

Parkinson’s disease as a synaptopathy

In PD and other neurodegenerative diseases, synaptic dysfunction is thought to be an early event that correlates with the manifestation of nonmotor symptoms [41], while later stages of the disease are characterized by neurodegeneration and cell loss [42–44]. Thus, synaptic dysfunction might be considered a prodromal stage of cellular pathologies that is then followed by axonal abnormalities, leading to the degenerative loss of neuronal somas [41,45–54].

aSyn-rich LB inclusions are present not only in neuronal cell bodies but also, and firstly, in axonal processes [55]. More recent studies reported aSyn in axonal dystrophic neurites in the striatum of PD [56] and DLB patients [57]. These studies suggest that synucleinopathy occurs in presynaptic terminals and axons, which goes in line with the predominant localization of aSyn in presynaptic terminals [4–6]. These early phenotypes can be attributed to the accumulation of toxic aSyn species within synapses, or the trapping of synaptic proteins by aSyn, ultimately leading to a process of dying back-like neurodegeneration.

Under physiological conditions, aSyn functions in its native conformation, possibly as a monomer. When the levels of aSyn start to increase, such as due to changes in its turnover, due to a decline in age-associated proteostasis or because of the effect of post-translational modifications [58,59], aSyn may gain abnormal properties and form toxic species. Among these toxic species, oligomers and fibrils are thought to play a key role in pathogenesis in synucleinopathies. These species can accumulate at presynaptic terminals, affect neurotransmitter release and synaptic transmission and, ultimately, cause synaptic dysfunction, leading to loss of neuronal connections and subsequent neuronal death (Fig. 1).

aSyn overexpression, which may potentiate the formation of toxic species, has been also shown to induce severe mitochondrial deficits, including fragmentation [31], mitophagy [60] and impaired mitochondrial protein import [24]. These are thought to be relevant for disease onset. Consistent with functional synaptic alterations at early stages of PD and other synucleinopathies, aSyn-induced mitochondrial impairment in the nigrostriatal system seems to start in synaptic mitochondria prior to the onset of generalized mitochondrial dysfunction in other parts of the neuronal cell.

aSyn interacts with the HSP10 chaperonin

In addition to the cytosol, aSyn has also been detected in mitochondria [61], where it affects, for example, respiratory chain complex activity [62,63]. aSyn interacts with several synaptic and mitochondrial proteins [64] and affects the function of these compartments [24,25,27,50,65–67].

Abnormalities in mitochondrial lipids and impairments in the electron transport chain were described in aSyn knockout mice. In addition, impairment in
mitochondrial complex I activity and increased ROS production in mitochondria are associated with the accumulation of wild-type aSyn in human dopaminergic neurons in culture and in postmortem PD brain tissue [63]. These findings suggest that both loss of function and aggregation of aSyn may affect mitochondrial function [62] and, thereby, affect neuronal homeostasis. Nevertheless, the exact impact of aSyn in striatal synaptosomal mitochondria is still unclear.

One of the proteins affected by aSyn is the 10 kDa heat shock protein (HSP10). HSP10 is a major hub protein in cellular interactions [68] that also plays an important role in regulating mitochondrial functions such as respiration, removal of ROS and maintaining mitochondrial membrane potential [69]. Together with the 60 kDa heat shock protein (HSP60), HSP10 forms asymmetric or symmetric complexes in mitochondria (Fig. 2). Interestingly, the asymmetric complex has decreased folding capacity, while the formation of the symmetric complex is limited by the levels of HSP10 [70]. A reduction in the function of HSP10/HSP60

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**Fig. 1.** Neurodegeneration associated with the accumulation of aSyn in presynaptic terminals. (A) Under physiological conditions, aSyn functions in its native conformation, possibly as a monomer, and localizes predominantly in presynaptic terminals. (B) Toxic aSyn species, generally known as oligomers and PFF, start to form and accumulate at the presynaptic terminal, leading to alterations in synaptic transmission and promoting synaptic dysfunction. (C) Ultimately, the accumulation of toxic species of aSyn leads to a process of dying back-like neurodegeneration.

**Fig. 2.** Schematic representation of HSP10/HSP60 complexes in mitochondria. The HSP10 forms either an asymmetric complex, assembled by one HSP10 heptameric ring (less efficient complex) (left), or a symmetric complex, assembled by two rings (right), with HSP60.
complex results in embryonic lethality [71], and a heterozygous mutation in the HSPE1 gene, encoding for HSP10, is associated with a severe, early-onset neurological disorder in humans [72]. Although the mutant protein retains some functionality, it leads to a reduction in the HSP10-to-HSP60 ratio. Since a reduction in the HSP10 levels results in the formation of asymmetric, less efficient complexes, the levels of client proteins, such as superoxide dismutase 2 (SOD2), decreased and, consequently, mitochondrial ROS levels increase [73]. Therefore, even small changes in the levels of HSP10 may alter the activity of multiple proteins and cellular functions. In the context of neurological diseases, the handling of HSP10 levels remains unclear [72,74–78]. Interestingly, we found that in either young or middle-aged aSyn transgenic animals, the levels of mitochondrial HSP10 are reduced, but the total striatal HSP10 levels were not affected by genotype or neither by age. In parallel, the levels of cytosolic HSP10 were raised in both young and middle-aged A30PaSyn mice [79]. Spare respiratory capacity (SRC), a measure of mitochondrial activity, is reduced in synaptosomes from young aSyn transgenic animals; however, the total mitochondrial fractions are not affected. Moreover, mitochondrial ROS handling, mitochondrial membrane potential and the opening of the mitochondrial permeability transition pore are also selectively compromised in the synaptic compartment of the striatum, but not in total mitochondrial fractions [79]. Both synaptosomal and total mitochondrial SRC are, however, reduced in middle-aged transgenic mice. These data suggest that age-associated decreased levels of mitochondrial HSP10 have functional implications.

Consistently, fractionation of putamen samples from PD patients and controls revealed that the levels of synaptosomal HSP10 and SOD2 are reduced in patients [79]. This causes an increase in mitochondrial 

![Healthy synapse](image1)

![Unhealthy synapse](image2)

**Fig. 3.** aSyn interacts with the HSP10 chaperonin in presynaptic terminals. Under physiological conditions, aSyn localizes predominantly in presynaptic terminals interacting with various proteins (left). The accumulation of toxic aSyn species leads to alterations in synaptic vesicles and to sequestration of HSP10 in the cytosol, preventing it from acting in the mitochondria (right). These alterations impair mitochondrial function and result in an increase in ROS and an impairment in ATP production, and ultimately leading to synaptic dysfunction.
oxidative stress and impairment of respiration, suggesting that HSP10 may be an important player in aSyn-associated mitochondrial dysfunction in synucleinopathies (Fig. 3).

Although there were no changes in soluble aSyn levels, HSP10 interacts with monomeric, oligomeric and preformed fibrils (PFFs) of aSyn with affinities in the micromolar range [79]. Considering that HSP10 is synthesized in the cytosol and that aSyn is also primarily a cytosolic protein, the primary site for the interaction between these proteins would be the cytosol. In our study, we showed that pre-incubation of PFFs with HSP10 reduces the folding activity of the HSP10/HSP60 complex. However, the folding activity of complexes already formed does not change [79]. Our study suggests conformation-specific interactions between aSyn and HSP10, given that we did not observe effects with aSyn monomers or PFFs.

Restoring the levels of HSP10 reduces aSyn pathology

As highlighted above, the link between aSyn pathology and mitochondrial dysfunction in PD is complex. Dysfunctional mitochondria produce less ATP and more ROS. If this takes place in the synapse, it likely leads to synaptic dysfunction [80] and, in turn, to the accumulation of aggregated aSyn [81–83] (Fig. 4). Mitochondrial health is essential for axonal transport, regeneration [84] and, consequently, to the clearance of aSyn [85]. This indicates that enhancing mitochondrial health might lead to delayed aSyn pathology and, as a consequence, to reduced aSyn-associated mitochondrial dysfunction.

Other chaperones, such as HSP27 [86,87], HSP70 [88–90] and HSP90 [91,92], have already been shown to play a role in aSyn aggregation and toxicity. Upon aSyn overexpression, the levels of HSP10 are reduced,
and overexpressing HSP10 restores the detrimental effects of WTaSyn, such as impairments in mitochondrial respiration and ROS handling. In WTaSyn-expressing cells, HSP10 overexpression results in increased levels of SOD2 and, consequently, in improved elimination of mitochondrial ROS. In addition, the mitochondrial serine protease high temperature-regulated A2 (HtrA2) levels and SRC are also normalized, suggesting an overall improvement in mitochondrial health. Interestingly, overexpression of HSP10 in cells expressing truncated forms of aSyn, thought to represent disease-relevant forms, improves mitochondrial ROS removal, but does not modify SOD2 and HtrA2 levels, nor does it improve SRC. This suggests that increasing the levels of HSP10 may enhance certain aSyn-associated mitochondrial impairments.

HSP10 overexpression also delays the relocalization of S129-phosphorylated aSyn (paSyn) from neurites towards the soma, consistently reducing the levels of insoluble aSyn [79]. These findings were confirmed in neuronal cells and also in vitro, using the real-time quaking-induced conversion assay [93,94]. Similarly, in aSyn transgenic mice, expression of HSP10 restores the levels of SOD2, decreases ER stress and reduces aSyn aggregation.

**Conclusion**

Herein, we provide our viewpoint on the hypothesis of the accumulation of toxic aSyn-aggregated species in synapses and axons prior to the onset of cellular pathologies associated with PD and other LB diseases. These aSyn aggregates promote neuronal dysfunction that can result in both intra- and extracellular toxicities. Intracellular mechanisms cause axonal degeneration, which progresses towards the cell body, eventually leading to neurodegeneration. Extracellular mechanisms may contribute to the spreading of aSyn species along synaptic connections and, ultimately, to the progression of synucleinopathy. Neither of these mechanisms is fully understood. One hypothesis, which we put forward, is that aSyn promotes the trapping of synaptic proteins, such as HSP10, as highlighted in our recent study. We propose that, like HSP10, other proteins may also be trapped by aSyn, promoting a vicious cycle, compromising their normal function and, thereby, destabilizing the normal neuronal physiology. In fact, and consistently with our viewpoint, recent studies have demonstrated that LBs may contain a variety of proteins and components that had not been previously appreciated [95], and that may be relevant for the alterations that result in neurodegeneration. Although we are still missing important information to fully understand the molecular mechanisms involved, studies focused on the idea that aggregated aSyn species may trap other biomolecules may bring new ideas that free us from older concepts that have failed, at least thus far, informing on novel targets that may lead to future therapies.

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**Conflict of interest**

The authors declare no conflict of interest.

**Author contributions**

PIS and TFO wrote the manuscript.

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