# Parkinson's disease and alpha-synucleinophaties: from arising pathways to therapeutic challenge

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**Abstract:** Parkinson's Disease (PD) and alpha synucleinopathies are multifactorial disorders, which manifest through motor symptoms and non-motor symptoms involving the Central Nervous System (CNS), the Peripheral Nervous System (PNS) and, recently, also the Enteric Nervous System (ENS). The typical hallmarks of these pathologies are known as Lewy Bodies (LBs) and Lewy Neurites (LNs), alpha synuclein (αS) proteinaceous, discovered in dopaminergic neurons of *substantia nigra* (*pars compacta*) as well as in other regions of the central and peripheral nervous systems. Despite the clear causes which lead to LBs/LNs are still unknown, according to Braak's theory, these inclusions appear first in PNS to spread, following neuronal innervation, towards the CNS. In line with these observations, several animal models have been used with the purpose to reproduce PD as well as to propose new therapeutic approaches. The present review highlights αS as the key-word for PD pathology and a main target in PD research, focusing on ER-stress and innovative immunotherapy, which could be a promising tool to reduce neuronal degeneration and to halt PD progression.

**Keywords:** Alpha-Synuclein; animal models; Braak's theory; endoplasmic reticulum; immunotherapy; mitochondria; Parkinson's disease.

## 1. INTRODUCTION

Parkinson's Disease (PD) is the second most common neurodegenerative disorder after Alzheimer's Disease (AD). Until now, two forms of the pathology have been discovered: an idiopathic form also named "sporadic Parkinson's disease" to indicate its unknown origin and a second genetic form also recognized as "Familial Parkinson's disease" [1]. Although the clear etiology is still unknown, degenerating neuronal populations in PD exhibits the accumulation of abundant intraneuronal inclusions known as Lewy bodies/Lewy neurites (LBs/LNs) containing the misfolded fibrillar protein alpha synuclein (αS) [2].

### 1.1. Principal features of αS protein

The αS protein is a 140 amino acid comprising peptide and a member of the synuclein family that also includes βS and γS. The amino acidic sequence of αS is characterized by an N-terminal amphipatic domain (1-60), a hydrophobic core denoted as non-Aβ component of Alzheimer disease amyloid (NAC, 61-95) and an acidic C-terminal negatively charged region (96-140) [3,4] (Fig. **1**).

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This protein is typically localized in the cytosol but the propensity of its N-terminal region to bind specific lipid rafts, enriched in cholesterol and sphingolipids, locates αS also in plasma membrane [5–8] as well as in sub-cellular compartments such as mitochondria [9]. The enrichment of αS in presynaptic terminals has led to the assumption that the protein is involved in the regulation of synaptic transmission, synaptic plasticity and neurotransmitter synthesis [10,11]. Moreover, in line with these observations, αS has been found to participate in the recycle of synaptic vesicles [12].

The native αS is unfolded, but under pathological conditions can undergo conformational changes, truncation, oligomerization, post-translational modification such as ubiquitination, hyperphosphorylation, nitration and oxidation and turns onto a β-sheet pathological structure, thus forming aggregates and becoming insoluble [13–15]. Point mutations (A30P, A53T and E46K) or gene amplifications of αS have been identified in familiar pedigree as responsible for the early onset of PD, suggesting that αS abnormalities are linked to pathogenesis of PD and other α-synucleinopathies [16–19]. Several studies have demonstrated that αS forms aggregates in a nucleation-dependent manner, where first soluble αS monomers forms soluble oligomers or protofibrils and, after a series of drastic conformational changes, they

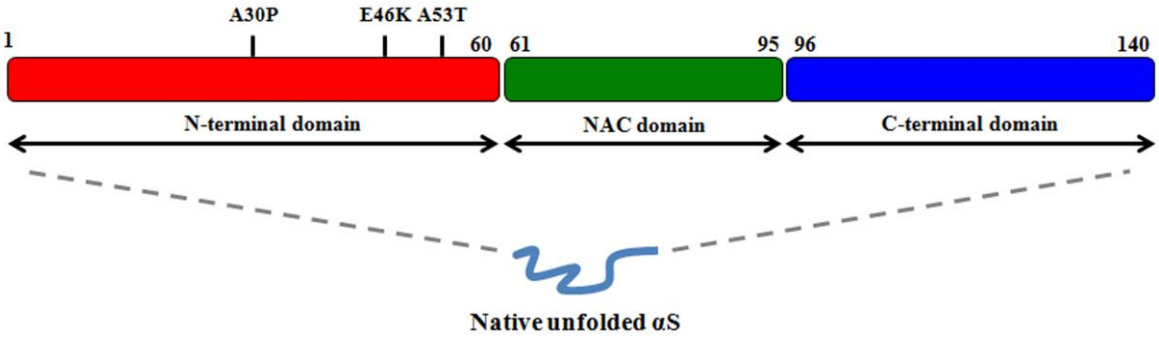


**Figure (1).** Primary amino acidic sequence of αS**.** The αS protein is made up by 140 amino acids. It is structurally characterized by three main regions: the N-terminal region containing the three point missens mutations (A30P, E46K, A53T) responsible of the early onset of PD also responsible for the binding with lipid membranes; the NAC central hydrophobic region important for the protein oligomerization and the C-terminal acidic region which can be subject of post translational modifications.

serve as nuclei to evolve into insoluble fibrils within cells [18]. It is not entirely clear which αS-aggregated form is cytotoxic and if the LBs/LNs are a cellular protective strategy to sequester αS aggregates avoiding the health cell impairment [20] (Fig. **2**). However, the hypothetical "toxic aggregates" are responsible for the unbalance between physiological and pathological αS proteins. In fact, an ever increasing body of evidence demonstrates that *in vitro* and *in vivo* formed αS monomer and fibrils are internalized by cells and may be propagated in a prion-like manner leading to endogenous αS aggregation and neurotoxicity [4]. Studies by Desplates and coworkers have shown how populations of human dopaminergic (DA) neuron cells (derived from SH-SY5Y neuroblastoma cells) were capable of taking up αS aggregates via endocytosis from neighboring neurons, forming, in turn, Lewy-like inclusions. Furthermore, these authors demonstrated that a directed host-to-graft transmission of misfolded αS occurs *in vivo* using a transgenic model of PD [21]. Other authors have also observed that the *in vitro* αS pre-formed fibrils (PFFs) are able to efficiently induce the aggregation and fibrillization of soluble endogenous αS in primary neuronal cultures generated from wild type (WT) mice [19,22,23]. It has been subsequently found that a single intra-striatal inoculation of pre-formed αS fibrils in WT non transgenic mice led to the cell-to-cell transmission of pathological αS, accumulation of intracellular LB/LN pathology and selective loss of DA neurons in anatomically interconnected regions [24]. Importantly, αS aggregates play another interesting role in triggering PD neurodegeneration. In particular, several studies have revealed that the transmission of neuronal αS aggregates takes place not only through a neuron-to-neuron interaction mechanism but also involving adjacent astrocytes. The αS astrocyte inclusions are probably addressed to a lysosomal sorting for their degradation [25,26]. Nevertheless, the persistence of neuronal αS aggregates provokes their accumulation in glial cytosol, triggering the release of pro inflammatory mediators such as cytokines and chemokines and inducing an inflammatory response as well as the activation of microglia, which is supposed to enhance neurodegeneration [27,28].

## 2. BRAAK'S THEORY

In agreement with this line, Braak and coworkers elaborated a theory based on neuropathological analysis of human brains of PD cases. According to these findings, initial LBs appearance occurs in locations no classically linked to PD such as the dorsal motor nucleus of the glossopharyngeal, vagal nerves and anterior olfactory nucleus and even the neuronal innervation of the gut. From those first locations, the LB pathology then progresses to higher brain centers following the vague innervation in a predictable ascending manner to the midbrain and finally to the basal forebrain and neocortex [29–32]. Detailed analysis of human brain samples allowed Braak and coworkers to distinguish six stages in the disease progression, including stages 1 and 2 where the pathology is still asymptomatic and LBs are spread only in peripheral areas, *medulla oblongata/pontine tegmentum*, in anterior olfactory structures and in the Enteric Nervous System (ENS). In stage 3 and 4, classical PD motor symptoms become apparent and the pathology reaches the midbrain, including *substantia nigra;* stage 5 and 6 are instead characterized by the involvement of neocortical regions in association with cognitive impairment [33–35]. Dissemination of LB pathology and neurodegeneration in areas outside the *substantia nigra* can also explain the incidence of non-motor symptoms that affect PD patients. Some of these symptoms, such as a reduced sense of smell, gastrointestinal (GI) dysfunction including salivation, dysphagia, impaired gastric

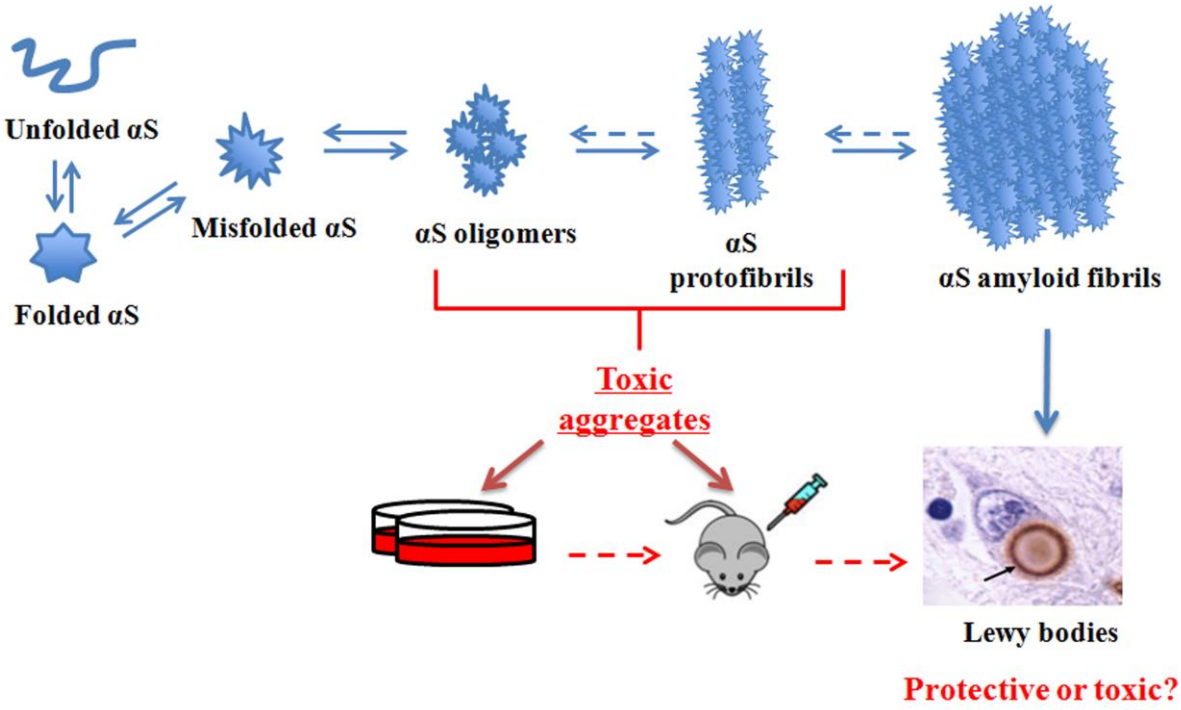


**Figure (2).** Hypotetical mechanism of αS amyloid fibrils formation and *in vitro/in vivo* trasmission of αS toxic aggregates. The native unfolded αS turns onto a folded or misfolded form having a high propensity of self aggregation into oligomers in a reversible process (double arrows). In a next step, oligomers continue to aggregate in a nucleation dependent manner until forming protofibrils and amyloid fibrils. In the latest process, reversibility is drastic reduced and misfolded αS is confined in the Lewis bodies. The αS oligomers and protofibrils could be the toxic aggregates, responsible for the cell-to-cell transmission of the Lewis bodies as demonstrated by *in vitro* and *in vivo* studies.

empting and defecatory dysfunction have been found to precede the development of somatic motor symptoms for decades before the disease's manifestation [36,37]. In fact, about 60% of PD patients have complained sustained constipation and GI dysfunction for long time before developing motor symptoms related to PD [38,39]. To confirm these findings, Holmqvist and coworkers have recently shown that different forms of αS from PD patient brain lysates, injected in the wall of the rat GI tract, in close proximity to the myenteric plexus, are transported retrogradely via the vagal nerves from the gut to the brain and that microtubule associated transport is involved in this propagation [40].

## 3. ANIMAL MODELS OF PD

Several animal models have been used to study PD, from unicellular eukaryote organisms such as the yeast *Saccharomyces cerevisiae*, or pluricellular ones as the nematode *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster*, to the evolutionarily similar non-human primates [41–43]. However, these models present obvious limits: yeasts or flies cannot suitably reproduce the loss of neurons in the brain typical of PD, while non-human primates present limitations in terms of costs and ethical concerns. Thus, because of their similarities to humans and these multiplicity of factors, rodent model is the most widely used to investigate PD [44]. To date, there are no rodent models that can reproduce completely the pathophysiology of PD. However, both the pharmacological and genetic models are frequently used not only to identify triggers and mechanisms of disease onset but also to develop strategies to stop neurodegeneration. The pharmacological model involves environmental toxins such as 6-hydroxydopamine (6-OHDA), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), paraquat or rotenone [45–47]. The second one is characterized by the transgenic over-expression of mutant autosomal dominant genes such as αS or leucine-rich-repeat kinase 2 (LRRK2), and knockout or knockdown models for autosomal recessive genes such as Parkin, DJ-1, PINK1 [48]. In particular, regarding the autosomal dominant mutations of αS gene, three missens point mutations have been identified in the early-onset of PD: A30P, E46K and A53T followed by the corresponding transgenic mouse models [49]. Specifically, Lee and coworkers have obtained an A53T αS

transgenic mouse model under the control of the mouse prion protein promoter (PrP). This model displays a robust neurodegeneration phenotype characterized by an adult onset of the pathology coupled with αS fibrillar accumulation in neurons and perikarya, astroglial reaction in affected brain regions and progressive motor dysfunction leading to death. Moreover, the same model is also associated with mitochondrial DNA damage, apoptosis of neocortical, brainstem and motor neurons [50]. Intriguingly, Kuo and coworkers have shown that A53T or A30P αS transgenic mouse models present ENS dysfunctions at a young age, before the onset of others non-motor symptoms such as the dysfunction in olfaction or changes in the brain stem and the dorsal motor nucleus of the vagal nerve (DMV) [51].

Thus, despite the pharmacological-based models are effective in showing a selective loss of DA neurons, they fail to replicate all the pathological features of PD such as the progressive changes in the brain and the onset of symptoms in the time. From this point of view, genetic models are more effective, they show a gradual progression of the pathology which allows to administer a therapeutic treatment prior the onset of the symptoms and to identify an hypothetical early biomarker .

## 4. THERAPEUTIC CHALLENGE

In the last years, scientific research on PD has focused his attention on identifying appropriate targets to which address new therapies. The protein αS seems to be the most promising key factor to investigate. Among the several therapeutic approaches, it could be farsighted acting on two levels (Fig. **3**):

- at the intracellular level, to reduce αS toxic species which probably impair the balance between the αS functional forms and toxic aggregates, these last interestingly considered in some studies as protective forms [52] (Fig. **2**);

- at the extracellular level, to circumvent αS cell-to-cell transmission and glial activation, leading to chronic inflammation, another pathological feature of PD [53,26].

### 4.1. ER/mitochondrial dysfunction and oxidative stress in PD: a considerable pathological pathway and a reasonable therapeutic target

The involvement of mitochondrial dysfunction in PD has been much dependent from the discovery of 1- methyl – 4 – phenyl- 1,2,3,6 – tetrahydropyridine (MPTP), followed by paraquat and rotenone, as potent and selective inhibitors of complex 1 of the respiratory chain. The use of these drugs result in the impairment of ATP synthesis and in the increase of oxygen free radicals (ROS), leading to oxidative stress [54]. Several mutations of mitochondrial DNA, encoding some respiratory chain factors, have been also correlated to complex 1 defects in PD [55]. Moreover, oxidative stress could be responsible for the accumulation of unfolded or mis-folded proteins such as αS at the intracellular level. In fact, the accumulation of mis-folded αS can disrupt the activity of another intracytoplasmic organelle, the Endoplasmic Reticulum (ER), involved in protein synthesis, glycosilation and folding, leading to the Unfolded Protein Response (UPR) [56,57]. This latest is a physiological protective response which stimulates a specific apoptotic pathway through the activation of the transcription factor C/EBP homologous protein (CHOP). The response also includes the inhibition of protein synthesis via the phosphorylation of the eukaryotic initiation factor 2α (eIF2α) [58–60].

To verify whether ER stress is involved in αS neurodegeneration *in vivo*, Colla and coworkers have reported that αS oligomers displaying the toxic conformation precede the onset of α-synucleinopathy and their accumulation in neuronal ER/mitochondria of humans as well as in A53T human αS mice, responsible of ER-stress induced apoptosis [61]. From these findings, the authors have hypothesized a possible evolution mechanism of αS abnormalities in ER/mitochondria: the αS flux toward the ER/mitochondria lumen increases with aging or the disease. It is made up by the presence of low initial levels of αS, then by immature soluble oligomers in the lumen, until they become detergent-insoluble macro aggregates/fibrils. The insoluble aggregates become exposed to the cytosol, probably by destabilizing the membranes, leading to ER stress, cell dysfunction and neurodegeneration (Fig. **3**). This hypothesis has also been confirmed by the use of Salubrinal, an anti-ER stress compound, which promotes dephosphorylation of eIF2α and increases CHOP, the downstream reporter for p-eIF2α. This treatment showed a decrease of ER/mitochondria associated toxic αS oligomers, a delay of the pathological onset and a life span extension of A53T αS mice [62,63].

In addition, Laguarta and coworkers explained the localization of αS in mitochondria – associated ER membranes (MAM), a sub-region of the ER that connects mitochondria to the ER, involved in cholesterol and phospholipid metabolism. Alterations in MAM-localized proteins or in MAM functions play a role in neurodegenerative disease. As reported by Laguarta: “in the case of PD, point mutations of αS resulted in its reduced association with MAM, a lower degree of apposition of ER with mitochondria and a decrease in MAM function causing an increase in mitochondrial fragmentation compared with WT αS”[64]. These findings are relevant because they have identified potential sites for a therapeutic intervention.

### 4.2. New frontiers in PD: the immunotherapy

An alternative and innovative therapeutic approach, which could be *ad hoc* to target “toxic αS aggregates”, uses immunotherapy. It has been widely applied for cancer, HIV, but recently his attention pointed towards neurodegenerative disorders. Several studies focus on Huntington, Alzheimer and Prion’s diseases, but a therapeutic intervention in α-synucleinopathies has been largely overlooked due to the pathological complexity and the uncertainty of the pathogenesis [65]. The use of immunotherapy in neurodegenerative disorders has been reinforced by the results from passive immunization against β-amyloid (Aβ) using monoclonal antibody (mAb) in AD, which has reached

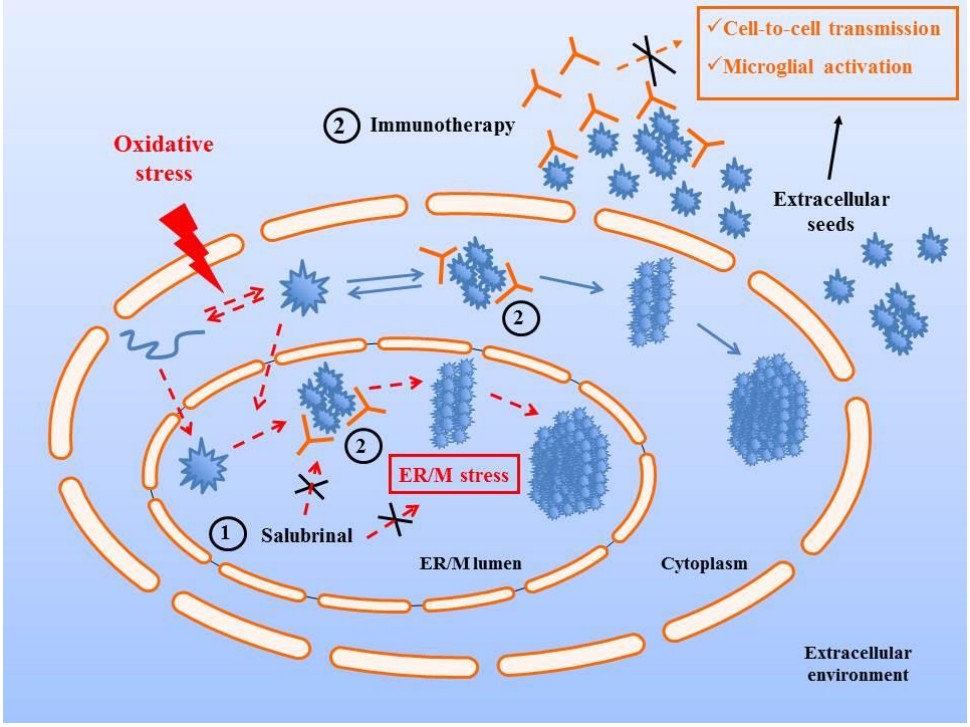


**Figure (3).** A model of proposed therapeutic strategies shown in cartoon form. The use of Salubrinal (1) reduces intracellular ER/M stress and the formation of toxic oligomers. Immunotherapy (2) (represented by mAb and intrabodies) can act on two levels: it provides the clearence of toxic aggregates present in the extracellular environment reducing their cell-to-cell transmission or the glial activation; it can also act intracellularly using intra-bodies which recognize specific conformational targets, modulating their effect.

phase III clinical trials [66]. Since Kohler and Milstein discovered the mAb technology [67], numerous advancements have been done passing through different engineered antibodies, until to discover the single chain antibody fragment (scFv). The use of scFv demonstrates multiple advantages including its application as intracellular antibodies or intra-bodies addressed to an intracellular target in order to modulate its function. Precisely, Meli and coworkers generated conformational sensitive antibodies with the aim of intercepting *in vitro* Aβ toxic oligomers (AβOs), crucial players in Alzheimer Disease (AD), in a subcellular compartment such as the ER. Of note, this tool shows a prominent aspect thanks to which is possible to target critical AβOs conformers inside the ER without interfering with the maturation and processing of the Aβ precursor protein (APP) [68,69].

As described previously, αS aggregates in PD have been associated with ER/mitochondria in human brain and in the A53T αS transgenic mouse model of PD. The use of a conformational intra-body against the αS toxic aggregates in a subcellular compartment could provide their clearance other than symptoms' improvement. Some studies [70–72] focused their attention on intra-bodies targeted directly to the brain, when the disease is already in an advanced neurodegenerative stage. However, the challenge could be to address conformational sensitive intra-bodies against αS to an early disease target such as the gut, when the pathology is still localized and the mis-folded αS is not widely spread in the CNS.

## CONCLUSION

The primary cause of Parkinson's disease is still unknown but numerous advancements have been done to a better understanding of the pathology. Despite the plethora of studies about the expression of αS, and the loss of DA neurons in the CNS, less information focused their attention on αS expression in the PNS or the ENS and its implications on patient's life. The studies mentioned in this review support the idea of using early biomarkers such as those localized in the GI tract, not only to anticipate PD diagnosis but also to intervene with innovative treatments contrasting the formation of αS aggregates and their cytotoxicity as well

as, according to Braak's theory, to avoid or to delay the ascendant progression of Lewy body's lesions to the CNS.

## CONFLICT OF INTEREST

The authors declare no conflict of interest as well as no financial contribution for this work.

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All authors significantly contributed to the manuscript drafting: FM has conceived, wrote and organized the manuscript; LB, LP and GG supervised the manuscript's draft, its English form and scientific coherence, approving the final version.

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