REVIEW

Research Priorities on the Role of α-Synuclein in Parkinson's Disease Pathogenesis

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ABSTRACT: Various forms of Parkinson's disease, including its common sporadic form, are characterized by prominent α-synuclein (αSyn) aggregation in affected brain regions. However, the role of αSyn in the pathogenesis and evolution of the disease remains unclear, despite vast research efforts of more than a quarter century. A better understanding of the role of αSyn, either primary or secondary, is critical for developing disease-modifying therapies. Previous attempts to hone this research have been challenged by experimental limitations, but recent technological advances may facilitate progress. The Scientific Issues Committee of the International Parkinson and Movement Disorder Society (MDS) charged a panel of experts in the field to discuss current scientific priorities and identify research strategies with potential for a breakthrough. © 2024 The Author(s). *Movement Disorders* published by Wiley Periodicals LLC on behalf of International Parkinson and Movement Disorder Society.

Key Words: α-synuclein; Lewy pathology; neurodegeneration; pathogenesis; protein aggregation

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α-Synuclein (αSyn) pathology is a major feature of Parkinson's disease (PD), and the occurrence of pathogenic variants in the *SNCA* gene[1-12] or multiplication of its locus[13,14] supports a possible causative role of αSyn in PD. Yet the nature of this role has long been a matter of debate due to unclear αSyn-mediated mechanisms of cell dysfunction, degeneration, and death. This is true not only for mutant αSyn but also for wild-type αSyn, which is expected to have a lesser effect on homeostatic mechanisms. Furthermore, it seems logical to question whether αSyn aggregation in the form of Lewy pathology (LP) constitutes a toxic insult leading to degeneration, represents an epiphenomenon that is not directly toxic but co-occurs with an alternative pathogenic process, or rather has protective functions for cells in a state of altered proteostasis.[15] Although none of these roles have been definitively demonstrated, data generally indicate that αSyn accumulation accompanies a decline in the function and health of neurons, either as a primary mechanism or secondarily by contributing to dysfunction originating from other mechanisms. This current dichotomy may be settled by integrating modern resources of experimental and clinical research. With potential disease-modifying therapies that reduce αSyn levels or pathology in the current drug discovery pipeline, a clearer understanding of αSyn's role in the pathobiology of PD is needed to develop the most effective therapeutic approaches. The Scientific Issues Committee of the International Parkinson and Movement Disorder Society (MDS) invited a panel of experts to discuss critical knowledge gaps regarding αSyn in PD focusing on six key questions (Fig. 1). Here, we summarize the most significant research recommendations at the modeling and clinical levels that may help to understand whether and how αSyn function/dysfunction plays a mechanistic role in PD.

What Are the Physiologic Functions of αSyn?

A better understanding of αSyn function and regulation in its native environment is required not only to support therapeutic strategies for reducing extracellular and/or intracellular αSyn or to favor native αSyn over its pathogenic states but also to understand the context in which disease develops. Loss of the normal function of αSyn does not appear to cause neurodegeneration, as demonstrated by multiple knockout studies in various cell and animal models, including triple knockout mice lacking α-, β-, and γ-synuclein and conditional knockout in adult and aging mice.[16-19] Others have reported toxicity with acute αSyn downregulation via adeno-associated virus short hairpin RNA in the rat nigrostriatal projection,[20,21] but none has been observed using other methods, including antisense oligonucleotides[22] and conditional knockout, which achieve near-total depletion of αSyn. The evidence thus suggests that loss of αSyn protein (and therefore normal function in its narrowest sense) does not produce

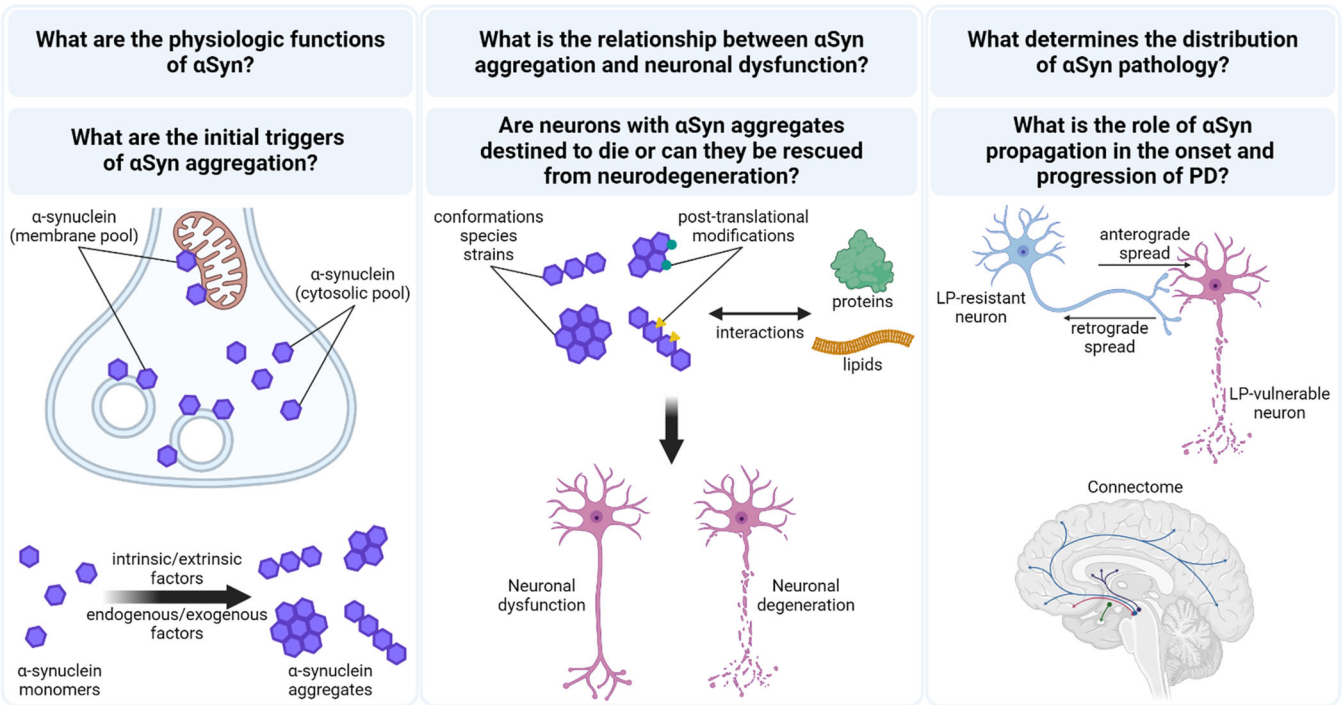

FIG. 1. Key questions to be addressed to identify research priorities on the role of (α-synuclein) in PD (Parkinson's disease) pathogenesis. [Color figure can be viewed at wileyonlinelibrary.com]

degeneration.[23] However, the normal function of αSyn remains highly relevant for degeneration because PD originates in the context of normal function. Thus, understanding transition to disease requires understanding normal αSyn function because disease may involve a gain in both normal and abnormal functions. The abundant membranous pathology in PD supports this possibility.

Membrane Binding

αSyn, a small protein enriched at presynaptic terminals,[24] is highly dynamic, cycling between a membrane-bound pool on synaptic vesicles and a cytosolic pool, and adopts multiple conformations in both states.[25] Membrane binding is mediated by its N-terminal sequence that forms a broken or continuous amphipathic α-helix upon lipid association and harbors most PD-linked mutations.[26-29] At rest, αSyn appears to associate with synaptic vesicles.[25,30] Upon neurotransmitter release, αSyn dissociates from this location,[25] but other than membrane curvature, factors that regulate this vesicular association remain largely unknown. Both N- and C-terminal sequences have been proposed to modulate αSyn membrane interactions through calcium binding, vesicle-associated membrane protein 2 (VAMP2)/synaptobrevin-2, β-/γ-synuclein, or synapsins.[31-35] αSyn multimerization and/or posttranslational modifications (PTM) may also influence αSyn behavior or function,[36-39] such as C-terminal phosphorylation at S129 (pS129), originally considered a hallmark of only LP. Neural activity has been shown to drive dynamic S129 phosphorylation, influencing protein–protein interactions at the synapse.[40,41] A better understanding of αSyn function will provide the context to elucidate the effects of these modifications and PD-associated mutations. Although membrane dynamics seem important, the specific roles of curvature, charge, and packing defects require elucidation. αSyn may also interact with other membranes, including mitochondria,[42,43] but the physiological or pathological significance of such interactions remains unclear. In addition, nuclear αSyn has been proposed to play a role in nucleocytoplasmic transport and DNA damage repair,[44-46] and its function may be impaired by aggregation. Furthermore, it remains unclear whether the cytosolic pool is functional or serves only a regulatory role. The mechanism of membrane association and interaction with cytosolic factors will shed more light on these questions.

Neurotransmitter Release

Due to its presynaptic location and presumed association with synaptic vesicles, αSyn has been considered to participate in neurotransmitter release, including behaviors of synaptic vesicle pools and direct effects on the fusion machinery. Yet αSyn is not an essential component for neurotransmission, supported by a subtle phenotype in αSyn deficiency models compared to severe phenotypes in deficiency models for proteins known to be essential for neurotransmitter release (eg, presynaptic soluble N-ethylmaleimide-sensitive factor attachment protein receptors [SNAREs], Munc18-1, and Munc13-1).[47] αSyn overexpression inhibits release possibly through multimerization, binding to VAMP2 and/or synapsin, and phase separation.[34,35,48,49] Inhibition of release does not appear to require aggregation. However, the normal role of endogenous αSyn has been less clear, including SNARE complex stabilization, regulation of the fusion pore, or endocytosis, but it seems to have modest effects on transmitter release.[31] Inhibition of release by overexpressed αSyn may thus involve a gain of function independent of aggregation. Yet αSyn aggregation can also result in reduced αSyn function by recruitment of physiologically active αSyn into pathological aggregates, as demonstrated by low levels of soluble synaptic αSyn on administration of αSyn fibrils in animals.[50-52] Interestingly, in songbirds, αSyn expression correlates with song acquisition,[53] suggesting a role in synaptic plasticity. αSyn levels upregulate in response to growth factors as well as multiple stressors,[54-57] and αSyn levels may not only initiate but also respond to pathogenesis.[58] In this regard, missense mutations (despite their low frequency) may provide insight into disease mechanisms. Several mutations affect membrane association,[59-64] although in different directions, suggesting multiple routes to disease that may involve shared and distinct molecular processes.[47]

Constitutive or conditional αSyn knockout does not cause neurodegeneration but results in hyperactivity and motor impairments, visual issues, and changes in presynaptic morphology.[17,18,31] Consistent with the possibility that functional αSyn may play a protective role in established PD, one study reported that PD patients with REP1 polymorphisms, which result in lower αSyn expression, had worse motor and cognitive outcomes once they develop PD,[65] although another smaller study reported the opposite results.[66]

Several important issues need to be addressed to shed light on αSyn function and regulation in its native environment. First, emphasis should be placed on physiological systems and models, examining αSyn function at endogenous levels in its native environment (in the central nervous system [CNS] and peripheral nervous system [PNS]) and in the nondiseased state. These native systems will enable the definition of biologically relevant lipid and protein–protein interactions in the native context of αSyn. Second, we need better tools to study the physiological functions of αSyn (eg, antibodies specific for cytosolic or membrane-bound αSyn and for physiologically relevant PTMs). Finally, we need to understand other functions of αSyn in the immune

response and its adaptive response to infections, which are likely to shed novel insight into its function in the brain.

What Are the Initial Triggers of αSyn Aggregation?

The mechanisms triggering αSyn misfolding and aggregate formation are still unclear.[67-69] Genetic, pathological, and molecular data support the idea that increased αSyn levels dominate the misfolding landscape, with protein buildup contributing to the aggregation pathway (oligomerization, fibrilization, and inclusion formation).[14,70,71] Conversely, mechanisms independent of protein levels might cause misfolding and aggregation. Existing data are inconclusive, but these alternative hypotheses do not seem to be mutually exclusive, particularly considering that αSyn accumulates in different genetic and sporadic forms of PD in both the CNS and PNS.[72] Clearly, studies aimed at determining whether basal protein level is a critical factor to switch from normal to misfolded conformation would provide novel insights into the mechanisms of aggregation. Taking a spatiotemporal perspective, we discuss molecular (intrinsic) mechanisms and primary causative mechanisms leading to αSyn misfolding and aggregation (Table 1).

Molecular Mechanisms

Several intrinsic factors of αSyn and its cellular environment may modulate the folding and misfolding of αSyn. Moreover, intrinsic factors (eg, PTMs, reduced clearance, and proteosome dysfunction) are likely impacted by environmental conditions.[73-75] It is important to consider that molecular mechanisms likely play a different role in each component of the aggregation pathway and cannot be taken out of the disease context. In the initial stages of protein misfolding, intrinsic factors might dominate because αSyn is an intrinsically disordered protein that adopts flexible structural ensembles[76] dependent on its surroundings. Higher protein levels requiring longer processing time would likely increase the probability of populating defined β-strand-rich structures that enter the aggregation pathway. αSyn conversion into misfolded forms may be a continuous process that, at some point and for unknown reasons (eg, impaired protein clearance due to age-related proteostasis dysfunction[77-79]), results in an overload of aggregation-prone αSyn. Mutations may change how αSyn is processed (folded and/or cleared) or the relation between membrane-bound and unbound protein, and thereby elevate its intracellular concentration.[32,80] Furthermore, underlying genetic or nongenetic factors may differentially alter regulation of other genes (eg, genes encoding for proteostasis network components), influencing molecular pathways and ultimately impacting the αSyn folding/misfolding landscape.

TABLE 1 *Triggering mechanisms for αSyn misfolding and aggregation*

Molecular mechanisms	*αSyn intrinsic factors*	• αSyn gene	• Missense mutations • *SNCA* multiplications • Single-nucleotide polymorphisms
		• Posttranslational modifications	
	Cellular environment	• Temperature, pH, redox status	
		• Molecular chaperones • Protein interactions • Proteostasis network changes	• Increased production (transcriptional and epigenetic regulation; translational-mRNA/ribosomal function) • Reduced clearance (chaperone-mediated autophagy)
Primary causative mechanisms	*Endogenous*	• Oxidative stress, elevated Ca^{2+} Metabolic/energetic changes Autoimmunity	
	Exogenous	• Infectious agents • Microbiome changes • Physical/head trauma	• Altered immune responses • Inflammation

Abbreviations: αSyn, α-synuclein; mRNA, messenger RNA.

Notably, transcriptional, posttranscriptional, translational, and posttranslational regulations of αSyn levels have been poorly studied in general, particularly in cell types that are most affected by αSyn aggregation (regional distribution) or that may have different mechanisms of protein regulation (glial cells).

Modeling is based on artificially induced aggregation, which may involve different mechanisms,[81-84] because spontaneous LP has not been reproduced even in genetic models that exhibit aggregation.[16] Notably, major questions about the misfolding/aggregation process for most αSyn mutants remain (eg, accelerated oligomerization with A30P mutation vs. increased fibrilization with A53T or E46K[85,86]). Although *SNCA* mutations are rare and, in some cases, associated symptoms differ from those in sporadic PD, the molecular effects of such mutations could provide insight into the misfolding/aggregation of wild-type αSyn. Therefore, it would be important to design novel studies using genetic models and to develop models of de novo αSyn aggregation, incorporating genetic/epigenetic, environmental, inflammatory, or other factors contributing to the terrain of protein misfolding. Some of these models have already been used, for example, genetic models with additional environmental toxicants such as pesticides[87-90] and models of αSyn aggregation facilitated by exposure to toxicants.[91,92] Because αSyn buildup in particular brain regions may be a major player in the aggregation process, models of region-specific conditional αSyn increase (preferably avoiding exaggerated αSyn levels) could help assess mechanisms leading to misfolding. Finally, using analytical approaches that start with studies of mechanisms triggering αSyn aggregation to characterize this common terminal pathway may help support or reject hypotheses about primary causative mechanisms.

Primary Causative Mechanisms

αSyn pathology may be caused by endogenous or exogenous mechanisms. Regarding endogenous causation, the localization of αSyn, which is largely presynaptic, membrane-bound,[93,94] and associated with membrane vesicle recycling, may increase demands for protein processing. αSyn turnover remains unclear, particularly at synapses and within axons, and whether the axonal capacity can suffice in a state of high demand is unknown. The somatic or axonal site of chaperone-mediated autophagy (CMA) that clears most αSyn in neurons also remains undefined.[95] Further, the distribution of αSyn pathology (brain region/cell-type specificity) is important to identify primary mechanisms of proteostasis network dysfunction, and time plays a role because αSyn pathology develops over many years, plausibly due to a gradual decline in proteostasis. Therefore, primary endogenous triggers for αSyn aggregation (eg, increased oxidative stress) need to be investigated considering cell-type specificities, subcellular compartments, and time requirements. Aged animals, especially nonhuman primates, are of particular interest due to differences in the misfolding landscape across species. Alternatively, epigenetic changes associated with aging can be applied to create an aged bioenvironment both in vitro and in vivo. Another possibility is to develop sufficiently aged human organoids that would allow for assessment of proteostasis machinery activity, in particular, human cell types.

Triggering αSyn aggregation may require the interaction of various mechanisms of cellular dysfunction instead of a single primary causative mechanism. For instance, metabolic/energetic impairment, which is favored by a particular bioenvironment (cell type and aging), may be more relevant in the context of altered proteostasis (overexpression or impaired clearance). It would be particularly helpful to develop research strategies taking a multitarget approach to test the combined effects of various endogenous mechanisms on αSyn folding/misfolding and aggregation.

Mechanisms associated with an identified exogenous cause lack supporting evidence, and most remain hypothetical. Some data of interest regarding infectious agents likely contributing altered immune responses, such as urinary tract infection[96] and SARS-CoV-2 infection[97] followed by increased protein load, have recently emerged. There is evidence that αSyn plays a role in the immune response to bacterial and viral pathogens (extensively reviewed[98]). Although acting as a restriction factor that inhibits viral transmission may be a normal function of αSyn,[99] it may also constitute another entry point for overloading the proteostasis network, ultimately leading to αSyn misfolding, buildup, and aggregation.

What Determines the Distribution of αSyn Pathology?

According to the Braak staging, LP in the CNS is hypothesized to spread from brain–environment interfaces (olfactory epithelium or gut), but recent work argues for both centripetal and centrifugal spreading.[100] In the elderly, LP seems to first occur in the olfactory bulb and only in a minority of individuals in the brainstem and more widespread in the periphery.[101] Regardless of the region first affected, it is unclear why LP distribution is restricted to specific brain regions through much of the disease course. To address this, the role of the brain connectome, the role of cell-autonomous factors, and the consequences of LP in vulnerable neurons need to be considered.

Brain Connectome

Both in vitro and in vivo studies have shown that synthetic αSyn pre-formed fibrils (PFF) can induce αSyn pathology (detected by pS129) spreading retrogradely (from postsynaptic host to presynaptic recipient)[102] and anterogradely (from cell body to axon terminal to postsynaptic recipient), arguing that LP propagation in humans could be bidirectional.[103-106] Although cell-to-cell spreading mechanisms remain controversial, they appear to be dominated by processes that do not involve any specific recognition of fibrillar αSyn (eg, macropinocytosis),[107,108] despite some studies suggesting receptor-mediated processes.[107] Some suggest fibril internalization through binding to heparin sulfate proteoglycans on the cell surface.[109,110] The mechanisms that mediate spread may be similar for naturally occurring αSyn aggregates (material extracted from human LP) and synthetic αSyn PFFs,[111,112] although unlike Alzheimer's disease pathology, LP does not collocate with heparin sulfate proteoglycans or glycosaminoglycans in PD brains as may be expected for prion-like internalization.[113] Given the bidirectional and nonspecific nature of these propagation mechanisms, the regional and cellular specificity of LP in PD brains is not clear. To solve this problem, several key gaps need to be filled. One is the generation of an unbiased, quantitative connectomic map of brain regions manifesting LP. Modern brain mapping approaches, like those advanced by the Allen Institute, can help but need to be systematically applied to structures implicated in PD. Relevant synaptic connections also need to be profiled for strength of the connection and their propensity to propagate misfolded αSyn. Finally, this mapping effort should consider animal species as an experimental variable.[114-116] Another gap is how the rules governing LP spread differ from the spread of PFFs. Spreading of synthetic PFFs differs from that of naturally occurring strains, which differ in conformation, resulting in unique biochemical and biological properties.[117,118] Finally, the role of region-specific factors in spreading needs to be determined. For example, inflammation has been implicated in LP propagation.[119] Regional variability in resident glia reactivity and accessibility to peripheral immune cells may contribute to the specific pattern of LP in humans. On the contrary, microglial macroautophagy may act to limit LP in certain regions.[120]

Cell-Autonomous Factors

Although LP propagation could be independent of cell type, its persistence and toxicity may be cell-type specific[121] and also dependent on αSyn expression levels, as shown in animal models.[122-124] Potentially relevant traits can be divided into several categories. First, do LP-vulnerable neurons rely more heavily on endocytic mechanisms leading to uptake of pathogenic forms of αSyn? Notably, neuronal spiking has been implicated in the regulation of micropinocytosis.[108] Neurons that have very high anabolic demands, like those with large axonal neurotransmitter release fields, may rely more on bulk uptake of extracellular proteins than more specific carrier-dependent mechanisms. Interestingly, one of the most vulnerable brain cell types, substantia nigra pars compacta (SNc) dopaminergic neurons, also releases neurotransmitters from their dendrites creating two subcellular regions where transmitter-related endocytosis is engaged. But it is unclear how common this is among vulnerable neurons. Second, do vulnerable neurons create an intracellular environment more prone to LP persistence and toxicity? To fill this gap, a systematic characterization of LP-vulnerable and LP-resistant neurons at the cellular/molecular level is required. Single cell-type-specific genomics, transcriptomics, epigenetic landscapes, and proteomics need to be compiled. Relevant functional studies using genetically encoded sensors for LP-relevant variables need to be conducted in appropriate contexts that mimic those in vivo. Ideally, these should be contexts in which neurons perform their normal functions (eg, physiological spiking, releasing neurotransmitter). For example, in vivo SNc dopaminergic neurons have a high rate of basal mitophagy, presumably because of bioenergetic demands associated with their network function.[125] This is unlikely to be recapitulated in vitro. Filling these knowledge gaps will also help determine to what extent LP is a consequence of trans-synaptic spread or de novo seeding within a vulnerable cell type.

Consequences of LP

For unclear reasons, some neuronal populations appear to tolerate LP without dying (eg, dorsal motor nucleus of the vagus [DMV] cholinergic neurons), whereas others succumb (eg, SNc dopaminergic neurons).[126] Why this is the case is unclear. It will be important to compare LP across regions at an ultrastructural/molecular level; in situ proteomics is an emerging field that should make this possible.[127] It is also possible that the functional impact of LP is cell-type specific. For example, pathogenic αSyn species compromise mitochondrial function[128-131]; if the spare bioenergetic capacity of a neuron is low, it may make αSyn-induced mitochondrial dysfunction intolerable. In this regard, it is necessary to determine how LP induces cell death in vulnerable cells. For example, neuronal death may involve mechanisms common to many cell types (eg, apoptosis) but have cell-specific triggers that should be explored experimentally. An obvious concern in these studies is how faithfully experimentally induced pathology recapitulates human pathology. Functional studies in humans, particularly those using imaging modalities to generate longitudinal data, will be important to include. These tools need to refine their

spatiotemporal resolution to generate reliable functional data from mesencephalic and other brainstem structures.

What Is the Relationship between αSyn Aggregation and Neuronal Dysfunction?

To understand αSyn's role in PD, it is important to evaluate the prevailing hypothesis that αSyn aggregation constitutes the toxic insult leading to neuronal dysfunction and death. Human genetics and neuropathology provide definitive evidence for strong links between αSyn aggregation and PD. However, it remains a challenge to prove a causal relationship between αSyn aggregation and neuronal dysfunction in humans; thus, model systems must be used and further developed.

Human Genetics

Pathogenic variants in *SNCA* or multiplication of its locus cause an aggressive clinicopathological syndrome of PD with high penetrance.[132] Although not exact replicas of sporadic PD, these rare cases suggest a central role of *SNCA* in PD pathogenesis given the clinicopathological similarities. Moreover, human genetics support a gain-of-function effect of αSyn, with a clear dose–response (triplication cases are more severe than duplication cases). Conversely, in population-based studies, individuals found to have a deletion of one *SNCA* allele lacked features of parkinsonism or dementia,[133] arguing against a pronounced loss-of-function effect of αSyn. Missense mutations alter the folding landscape of αSyn, thus affecting its propensity to aggregate as well as its interactions with protein partners and lipids/membranes. Whereas many amino acid residue substitutions within αSyn are clearly associated with increased aggregation, substitutions not associated with αSyn pathology certainly exist but are not necessarily considered. Genome-wide association studies (GWAS) have clearly indicated *SNCA* as the most important locus separating sporadic PD from controls, and single-nucleotide polymorphisms (SNP) within the *SNCA* locus have been linked to earlier age of onset. These GWAS findings highlight the utmost significance of the *SNCA* locus for disease manifestation and its evolution, for not only familial but also sporadic PD. There remains a need to better understand how disease-associated mutations change protein structure, alter physiological properties, and confer toxicity, whether αSyn aggregates or not, especially as additional *SNCA* variants are identified. More prospective studies are also needed to follow the trajectory of those with "deleterious" *SNCA* SNPs who may have a more aggressive disease course. Such research will more clearly delineate αSyn as a risk factor, instigator, and/or driver of the disease process.

Human Pathology and Model Systems

There has been some controversy about whether αSyn levels are overall increased in PD or just shifted toward more insoluble conformations. Using isolation by laser-capture microdissection, *SNCA* messenger RNA (mRNA) levels have been shown to be significantly increased in sporadic PD, compared to controls, in individual remaining nigral tyrosine hydroxylase (TH)-positive neurons, albeit from a small sample of brains.[134] *SNCA* mRNA levels are also increased in human induced pluripotent stem cell (iPSC)-derived dopaminergic neurons upon seeded aggregation of endogenous αSyn with PFFs.[58] This suggests that aggregation upregulates αSyn expression, which in turn favors aggregation, leading to a self-maintained pathogenic cycle. It will be crucial to extend these studies to include quantification of αSyn protein and identification of αSyn conformation(s) at the individual cellular level in cases across the pathological continuum of PD, including very early stages (eg, prodromal/premotor PD, incidental Lewy body disease [iLBD]).

Findings from human pathology suggest that diffuse accumulations of αSyn in the form of pale bodies or pale neurites may form first,[135-138] evolving into more dense structures that eventually assume the compact structure of mature LP, containing not only αSyn fibrils but also cellular chaperones, membrane proteins, lipids, organelles, and cytoskeletal elements. Toxicity may result from this process. For instance, sequestration of proteins within LP may result in functional deficits, leading to cell stress and death.[139] In addition, αSyn aggregates can redistribute important lipid and protein components of neuronal plasma membranes,[140,141] which may be deleterious to neurons.[142] In experimental models, a range of proteins, vesicles, and organelles are sequestered within mature Lewy body–like structures. Lewy neurites may also disrupt axonal transport and dendritic spines,[143-148] thereby impairing the ability of neurons to properly process synaptic information or convey that information to other neurons. Thus, LP may compromise a neuron's function without causing degeneration, although neuronal terminals/synapses may be primarily affected. Given that LP may be present in neurons for years, this is of potential therapeutic relevance.

LP may not always be necessary for neuronal dysfunction or neurodegeneration. For example, in a small cohort of patients classified as having Braak stages 1 and 2 (ie, before LP involves the SNc), approximately 12% cell loss was already observed in the SNc[149]; however, it must be noted that nigrostriatal terminals, which may already harbor LP, were not assessed and

that these findings have not yet been replicated in a larger cohort. Furthermore, severe nigral degeneration and levodopa-responsive parkinsonism can occur in the complete absence of LP. This is best exemplified by the majority of parkinsonian patients with biallelic mutations in *PRKN* and an appreciable minority of cases with *LRRK2* mutations.[150] These genetic forms may be unrelated to sporadic PD. Even so, LP absence does not exclude a pathogenic role for other αSyn conformations. For example, proximity ligation assays can visualize presynaptic αSyn disease-associated oligomers,[151] which may be more robustly correlated with neuronal dysfunction than LP.[152] It will be important to develop tools able to detect various conformations of αSyn in postmortem tissue and to visualize their formation in living neurons and other cells in model systems. The latter will allow for correlations to be made with changes in cell function in real time. Specific identities and vulnerabilities of cell types will need to be considered. In parallel, binding partners of αSyn in this gradual process will need to be determined. Such work will broaden our understanding of why certain neuronal populations and circuits appear to be impervious to heavy LP loads, whereas others are extremely sensitive.

What Is the Role of αSyn Propagation in the Onset and Progression of PD?

Although postmortem studies using routine immunohistochemistry suggest αSyn aggregation may first occur in the olfactory bulb, autonomic nervous system, or enteric structures, we still do not know specifically where in the CNS or PNS disease begins. Moreover, there are likely clinical subtypes of PD that differ in this respect (and others) that are not yet adequately distinguished or characterized. For example, studies using αSyn seed amplification assays can identify disease-associated seeding activity in patients with manifest PD,[153-156] as well as individuals with prodromal/premotor PD, such as idiopathic rapid eye movement sleep behavior disorder[157] and pure autonomic failure.[158] However, it is unknown how early in the disease process these methods can detect abnormal αSyn, or if its detectability is subtype dependent. These assays are currently unable to serve as quantitative biomarkers of disease progression. Similarly, apart from imaging the presynaptic nigrostriatal dopaminergic pathway, we have no reliable measures of disease status that can be used to quantify and monitor ongoing neuronal dysfunction or progressive neurodegeneration. Without robust tools for detecting and measuring pathogenic αSyn, there are several challenges to determining its role in onset and progression of PD in both experimental and clinical research.

Experimental Research

Identifying the anatomical location(s) where pathology starts is expected to improve experimental models of PD by defining potential sites of injection for αSyn seeds to recapitulate most faithfully prion-like pathology progression. However, these models often rely on phosphorylated αSyn (usually pS129) to detect and monitor spread of pathogenic protein. This approach relies on several assumptions, notably that all pathogenic αSyn is phosphorylated at one specific residue. Yet in vitro and in vivo studies have shown that pS129 is not necessary for the formation of pathogenic αSyn.[159,160] It is increasingly recognized that αSyn misfolding and propagation long precede the formation of pathological inclusions that are detectable via traditional immunohistochemical methods.[151,152,160-163] Several cell-based and cell-free assays now exist for identifying disease-associated αSyn conformations. However, there are limitations in their use to study onset, and they cannot be used to evaluate spread of disease in vivo. Thus, improved research tools that are not antibody dependent to identify the presence of pathogenic αSyn are needed.

A prevailing hypothesis is that disease progression results from prion-like spread of αSyn. Importantly, experimental models typically used to investigate this process rely on various αSyn fibril strains, including patient-derived and synthetic strains. Decades of research on prion protein have shown that the strain adopted by the misfolded protein determines its biochemical properties and spreading kinetics.[164] Thus, two different strains may spread to the same brain regions in an experimental model but with different time frames. For example, if the distribution of αSyn neuropathology is assessed 4 months after two different strains are injected into the brain, spread of either strain to a particular brain region is impacted by its rate of replication. If the same titer (ie, amount of replication-competent αSyn, which is not the same as total αSyn) of each strain has not been used, it is impossible to determine if the presence or absence of pathology in a brain region is due to strain biology or differences in titer. Given the growing structural studies identifying unique αSyn strains,[165-179] the field needs to develop operational definitions of titer for each αSyn strain in use. Moreover, although synthetic strains share some structural characteristics with fibrils purified in the presence of detergents from patient brain homogenates, including a Greek key motif, they fail to fully recapitulate all structural properties, including protofilament interfaces, intermolecular interactions (eg, residues involved in salt bridges), cofactors, and protofilament number and symmetry.[165,166,168-170,172,175-178,180,181] Despite remarkably triggering pathology, varied fibril sources

and preparations across laboratories impede comparing strain-specific findings as they pertain to PD pathogenesis. Further, it is unclear what data generated using synthetic strains are disease relevant. We suggest standardizing experimental conditions to evaluate αSyn spread, including (1) operational definitions and consistent methods for isolating and injecting αSyn fibrils and (2) developing methods for faithfully amplifying disease-relevant structures from biomaterials (eg, brain homogenates, cerebrospinal fluid).

Clinical Research

It is well established that LP, as the current marker of pathologic αSyn, is not sufficient for onset of neurodegeneration. iLBD is found in approximately 15% of seemingly healthy elderly individuals,[182-185] with no clear evidence for widespread neurodegeneration. Furthermore, in PD patient brains, there are several regions that demonstrate considerable αSyn aggregation with little cell loss, including the cingulate cortex, DMV, and, early on, locus coeruleus.[186-190] This raises an important question regarding the extent to which αSyn pathology is inducing neuronal dysfunction in the absence of neurodegeneration. This may be as important to the patient as the consequences of neuronal loss. It is also important to establish whether dysfunction inevitably evolves to neuronal loss and whether dysfunction can be reversed with appropriate treatment.

Critical to understanding αSyn's role in PD progression is defining and assessing this process. Importantly, progressive worsening of clinical parkinsonism is not necessarily indicative of progressive neurodegeneration and, even when this is the case, it may not be due to further propagation of pathogenic αSyn. Progressive neuronal dysfunction, whether or not initially triggered by αSyn, could occur due to distinct pathobiological processes that no longer require αSyn. A similar phenomenon could be postulated for progressive SNc neurodegeneration. Clinical progression, especially in early PD, is generally assessed by worsening of levodopa-responsive motor features, presumably indicative of progressive striatal denervation. However, it is unknown whether this is a consequence of active spreading of pathogenic αSyn driving SNc neurodegeneration. Alternatively, it is possible that once pathogenic αSyn has impacted the SNc, likely through cell-to-cell prion-like spread, other critical pathobiological processes are triggered and capable of independently driving the neurodegenerative process.[191] If this is the case, subsequent disease progression related to further αSyn propagation might be clinically evident only as other brain regions become affected (eg, progressive cognitive decline, development of other nondopaminergic manifestations). Thus, there is a critical need for reliable biomarkers to detect and quantify the presence and spread of pathogenic αSyn in living patients, as well as more sensitive and broadly based biomarkers for both neuronal dysfunction and neurodegeneration.

Are Neurons with αSyn Aggregates Destined to Die, or Can They Be Rescued from Neurodegeneration?

αSyn aggregates are observed in several brain regions not associated with significant cell loss, suggesting not all neurons with αSyn aggregates are destined to die. However, how the biochemical and structural properties of αSyn in these regions compare to αSyn aggregates in regions undergoing substantial cell loss is poorly understood. Greater understanding requires recognizing and capturing αSyn aggregate diversity in PD longitudinally to examine the time course of αSyn aggregation and neuronal death.

Diversity of αSyn Aggregates

αSyn pathology requires the formation of αSyn aggregates, including fibrils, which constitute the predominant proteinaceous aggregates found in LP. Immunohistochemical characterization of αSyn aggregates in postmortem brains has revealed highly diverse structures ranging from puncta to diffuse aggregates, to highly condensed eosinophilic structures with radiating filaments, and other types of inclusions composed of highly complex mixtures of membranous organelles, lipids, and dispersed fibrils.[192] These different types of αSyn aggregates could represent (1) different species on the pathway to LP formation or distinct types of αSyn aggregates, (2) αSyn aggregates that arise from different aggregation pathways or cellular interactomes, or (3) aggregates that were differentially modified and remodeled as part of the cellular response to neutralize their activity. This requires that we move away from defining αSyn aggregates based on generic tools that are not capable of capturing this diversity. For example, antibodies against pS129 αSyn or amyloid-binding small molecules, such as Thioflavin T/S or Congo red, remain primary tools for detecting and quantifying αSyn pathology in postmortem human brains and preclinical models of PD, despite neither recognizing αSyn oligomers nor capturing biochemical diversity of pathology. Developing αSyn oligomer-specific antibodies is possible but has so far proven to be challenging. Another challenge is to develop antibodies that distinguish between dynamic physiological oligomeric forms of αSyn from nonnative and potentially pathogenic oligomeric forms, especially given our lack of knowledge of biochemical and structural properties of native oligomers. Similarly, developing new

tools to monitor changes in conformational properties of αSyn is challenging because we lack knowledge about the native conformation of αSyn in biological membranes and different cellular compartments. Developing antibodies targeting the membrane-bound state of αSyn may be possible using αSyn bound to synaptic vesicles as antigens.

Biochemical modifications of αSyn aggregates can alter their effects on neuronal function and survival. For example, recent studies indicate that certain PTMs

TABLE 2 *Top research priorities recommended by the panel*

Recommendations	Strategies/approaches
Applying physiological systems/models to characterize αSyn function at endogenous levels in native environment and nondiseased state	• Targeting interactions with lipids and proteins, function in cellular compartments, and role in the immune response
Determining the impact of disease-associated mutations on αSyn function and fibril structure	• Analyzing changes in protein structure, physiological properties, and toxicity
Conducting systematic analyses of αSyn regulation and the role of αSyn levels	• Targeting from transcriptional to posttranslational regulation with cell-type specificity • Using available genetic models and conditional models for region-specific αSyn buildup
Considering cell-type specificity, cell compartment, and time requirements for studies of triggering mechanisms of αSyn aggregation	• Using aged animals (including aged nonhuman primates) and aged human organoids • Modeling epigenetic changes related to aging
Developing cell and animal models that recapitulate the different stages of αSyn aggregation	• Incorporating the processes of oligomerization, fibrillization, and inclusion formation
Developing de novo animal models of αSyn aggregation	• Incorporating environmental, inflammatory, and other contributors to misfolding
Designing multiple target studies of primary mechanisms of αSyn folding/misfolding and aggregation	• Targeting the interaction of various mechanisms (eg, oxidative stress, elevated Ca^{2+}, metabolic/energetic changes)
Systematically characterizing LP-vulnerable and LP-resistant neurons at the molecular and cellular levels	• Applying genomics, transcriptomics, epigenetics, proteomics, in situ (spatial) omics, and functional studies in the context of in vivo cell function using LP-relevant sensors
Generating an unbiased, quantitative connectomic map of LP distribution	• Including number and strength of synaptic connections, considering animal species differences
Establishing standardized experimental approaches for evaluating αSyn spread using in vivo models	• Obtaining consensus on operational definitions and common methods for isolating and dosing with disease-relevant αSyn fibrils • Developing methods for faithfully amplifying disease-relevant structures from biomaterials, such as brain homogenates or CSF
Developing better tools to label αSyn in its various forms and cellular locations	• Including antibodies, ligands, sensors, and other markers for application to model systems and human brain tissue
Analyzing cellular responses to LP in models and humans	• Targeting functional readouts at the single-cell and population levels (eg, metabolic changes) • Applying longitudinal imaging studies with refined spatial and temporal resolution
Developing reliable biomarkers to detect and quantify the presence and spread of pathogenic αSyn in patients across the disease spectrum (from premotor to advanced PD), and more sensitive and broadly based biomarkers of both neuronal dysfunction and neurodegeneration	• Considering various modalities of biomarkers (ie, chemical, structural, functional) • Conducting large prospective studies to define the natural history of individuals with positive αSyn biomarkers (eg, positive αSyn seed amplification assays) and the factors that influence the development of clinical disease
Prospectively studying the trajectory of people with "deleterious" *SNCA* SNPs who may have a more aggressive disease course	• Conducting longitudinal cohort studies

Abbreviations: αSyn, α-synuclein; LP, Lewy pathology; CSF, cerebrospinal fluid; SNP, single-nucleotide polymorphism.

can exert strong effects on modulating αSyn fibril seeding activity. Two recent studies showed that nitrated or O-GlcNAc-modified αSyn fibrils exhibit minimal or no seeding activity in primary neuron cultures and seeding-mediated mouse models. This work assumes that PTMs occur prior to templated misfolding, which may not be the case, as others have shown that replication of pathogenic αSyn occurs prior to PTM addition.[193,194] Interestingly, a recent study demonstrated that truncated αSyn is present in astrocytes SMP. However, whether this represents a pathogenic species or simply glial-mediated clearance of fibrillar αSyn remains unknown. These findings indicate further investigation of the role of PTMs in αSyn pathogenesis is needed. Although several PTMs have been consistently observed in pathological αSyn aggregates isolated from PD brains (eg, phosphorylation [Y39, S87, S129, Y125], nitration [Y39, Y125], ubiquitination at multiple N-terminal lysine residues, N- and C-terminal truncations), more systematic and quantitative mapping of αSyn PTMs at different stages of disease progression is essential to identify and prioritize disease-relevant PTMs that could be targeted for biomarker and therapeutic development.

Time Course of αSyn Aggregation and Neurodegeneration

To determine whether accumulation and/or aggregation of αSyn in neurons is sufficient to induce neurodegeneration, one must be able to conduct longitudinal single-cell tracking studies where induction of αSyn aggregation and formation of LP-like aggregates and cell death occurs within a defined and reasonable period. This is not currently possible in human brains, and the only preclinical models that enable such studies are the PFF seeding-based models of αSyn spreading, which do not fully recapitulate PD pathology. Given the lack of αSyn imaging agents that allow direct monitoring of this process, studies in cellular and rodent models expressing a fluorescent form of the protein enable monitoring αSyn aggregation kinetics while also following the fate of neurons containing αSyn aggregates. One limitation of these studies is their reliance on αSyn fused to fluorescent proteins, which can modify the surface of fibrils and alter their interactome. Interestingly, uptake of αSyn fibrils into neurons that do not express αSyn does not induce neuronal dysfunction or death, whereas internalization of αSyn into primary neurons induces αSyn fibrillization, formation of αSyn inclusions, and neuron loss. These observations suggest that the process of αSyn seeding and fibril growth, rather than mere uptake or presence of αSyn aggregates in neurons, is the primary driver of αSyn toxicity and cell death.

We propose that any framework for investigating the relationship between αSyn pathology formation and neurodegeneration should (1) critically consider multiple scenarios that capture the complex relationship between αSyn aggregation, pathology formation, and neurodegeneration; (2) include clear working definitions of the different types of αSyn species and consensus on outcome measures to assess neuron dysfunction and death; (3) account for differences in αSyn protein expression; (4) account for the diversity of αSyn aggregates/pathology observed in PD brains; (5) consider the possibility that not all αSyn aggregates are pathogenic (ie, some aggregates are toxic and/or seeding competent and others not); (6) acknowledge that differences in the kinetics of αSyn aggregate formation and clearance could be key determinants of the final fate of affected neurons; (7) consider the possibility that transient αSyn aggregation could trigger pathogenic pathway and cascading events that, once initiated, become independent of αSyn aggregation; and (8) accept that neurodegeneration could result from both aggregation-dependent and aggregation-independent mechanisms. It is important to develop preclinical models that recapitulate the different stages of αSyn aggregation formation and tools that enable specific detection of these pathologies. These resources would allow for testing established and new hypotheses and for dissecting the role of different αSyn species and the process of αSyn aggregation and LP formation in the development of PD and other αSyn-related disorders.

Conclusions

This discussion of knowledge gaps regarding αSyn's role in PD has identified major barriers and priorities for research progress. It is clear that lack of more suitable in vivo models and probing tools is a critical issue across molecular, cellular, and physiological studies. It has also been underscored that recent work contributed mostly incremental progress by expanding on similar approaches. The perspective of the panel is that developing new models/tools and refining the strategy-to-target design are important to advance our understanding of αSyn function/dysfunction and its involvement in PD pathogenesis. Table 2 summarizes the panel's recommendations, reached by consensus, for top research priorities. ■

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Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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APPENDIX

The MDS Scientific Issues Committee:

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SMP, DJS, LVK, and RT contributed to the conception and design of this article. All authors contributed equally to data analysis and drafting the manuscript according to the panel discussions. All authors also contributed equally to reviewing, editing, and revising the manuscript.

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J.B. reports no disclosures.

R.H.E. is on the advisory board of Nine Square Ventures.

G.H. owns stocks in Cochlear Inc and NIB Holdings; is a consultant for NHMRC and the Australian Government; is employed by the University of Sydney; receives royalties from the Academic Press, Elsevier, and Oxford University Press; receives grants from NHMRC, NHMRC-EU Joint Program on Neurodegenerative Disease, Aligning Science Across Parkinson's (ASAP), NIH (RO1), The Michael J. Fox Foundation, Shake-it-up Australia, and University of Sydney.

A.E.L. has served as an advisor for AbbVie, AFFiRis, Alector, Amylyx, Aprinoia, BlueRock, Biogen, BioAdvance, Biohaven, BioVie, BMS, CoA Therapeutics, Denali, Janssen, Jazz, Lilly, Novartis, Paladin, Pharma 2B, PsychoGenetics, Retrophin, Roche, Sun Pharma, and UCB; received honoraria from Sun Pharma, AbbVie, Paladin, and Sunovion; received grants from Brain Canada, the Canadian Institutes of Health Research, the Edmond J. Safra Philanthropic Foundation, the Krembil Brain Institute, The Michael J. Fox Foundation, the Ontario Brain Institute, the Parkinson Foundation, Parkinson Canada, and the W. Garfield Weston Foundation; is serving as an expert witness in litigation related to paraquat and Parkinson's disease; and received publishing royalties from Elsevier, Saunders, Wiley-Blackwell, Johns Hopkins Press, and Cambridge University Press.

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