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Clearance Pathways for α-Synuclein in Parkinson's Disease

Margaret S. Ho[1,2]

[1]Institute of Neuroscience, National Yang Ming Chiao Tung University, Taipei, Taiwan | [2]Brain Research Center, National Yang Ming Chiao Tung University, Taipei, Taiwan

Correspondence: Margaret S. Ho (margaret.ho@nycu.edu.tw)

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ABSTRACT

Protein aggregation and accumulation are hallmark features of neurodegenerative diseases. In Parkinson's disease, the progressive formation and propagation of α-synuclein aggregates—found in Lewy bodies and Lewy neurites—are closely linked to widespread neuronal dysfunction, dopaminergic neuron loss, and the emergence of both motor and nonmotor symptoms, including anosmia, cognitive decline, and depression. Despite their pathological significance, the mechanisms underlying the formation, spread, and clearance of these aggregates remain incompletely understood. In this review, we examine the cellular and molecular pathways responsible for the elimination of protein aggregates in the diseased brain. We first summarize various experimental models of α-synuclein pathology, followed by a discussion of the degradation mechanisms in neurons and glial cells under pathological conditions. These findings offer new insights into cell type-specific clearance pathways and highlight potential therapeutic targets for mitigating α-synuclein–associated toxicity in Parkinson's disease.

1 | Introduction

Second to Alzheimer's disease, Parkinson's disease (PD) is the most prevalent neurodegenerative disorder, affecting approximately 1% of individuals aged 65 and older (Kouli et al. 2018; Aarsland et al. 2021). First described by James Parkinson in his 1817 essay *An Essay on the Shaking Palsy* (Parkinson 2002), PD is characterized primarily by motor dysfunction, including bradykinesia, resting tremor, postural instability, and muscular rigidity. Moreover, a wide spectrum of non-motor symptoms—including sleep disturbances, constipation, depression, anxiety, and cognitive decline—as well as cranial sensorimotor manifestations such as voice and swallowing dysfunction, are also commonly observed (Lee and Koh 2015; Pfeiffer 2016; Kouli et al. 2018; Fleming et al. 2012; Lewis and Byblow 2002). These multifaceted clinical features are cultivated from different cellular defects in the brains, one of which is the progressive loss of dopaminergic (DA) neurons in the *substantia nigra pars compacta* (*SNpc*). In correlation with the clinical symptoms, DA neurons degenerate in a sequential pattern across different brain regions, indicating that both the propagation of pathology and region-specific vulnerability contribute to the development of PD (Braak et al. 2003; Giguère et al. 2018; Prell 2018). Notably, beyond dopaminergic neurodegeneration, cholinergic neurons

Abbreviations: α-syn, α-synuclein; AAV, adeno-associated virus; ALP, autophagy-lysosome pathway; ATG1/ULK1, ATG1/UNC-51-like kinase 1; ATGs, autophagy-related genes; CMA, chaperone-mediated autophagy; DA, dopaminergic; dAux, dAuxilin; ER, endoplasmic reticulum; GAK, Cyclin-G-associated kinase; LAMP2A, lysosome-associated membrane protein 2A; LBs, Lewy bodies; LRRK2, leucine-rich repeat kinase 2; Nrf2, erythroid 2-related factor 2; PD, Parkinson's disease; PFF, α-syn preformed fibrils; PI3K, phosphatidylinositol 3-kinase; PINK1, PTEN Induced Kinase 1; SNpc, substantia nigra pars compacta; UPS, ubiquitin–proteasome system.

in the basal forebrain (Müller and Bohnen 2013; Perez-Lloret and Barrantes 2016) and the locus coeruleus–norepinephrine system (Del Tredici and Braak 2013; Sun et al. 2023) are also compromised across different stages and brain regions of PD.

Another hallmark of PD pathology is the accumulation of Lewy bodies (LBs) and Lewy neurites—cytoplasmic inclusions primarily composed of aggregated α-synuclein (α-syn) (Spillantini et al. 1997; Baba et al. 1998; Spillantini et al. 1998). Similar to the pattern of DA neuron loss, the anatomical spread of LBs correlates with symptom progression, forming the basis for the Braak staging hypothesis (Braak et al. 2003). Despite the well-documented association between LBs and disease severity, the mechanisms underlying LB formation, propagation, and clearance remain incompletely understood. Whether their removal slows disease progression is still under debate, highlighting the importance of dissecting LB pathology in understanding PD pathogenesis.

Both familial (monogenic) and sporadic forms of PD have been recognized as arising from a complex interplay between genetic predispositions and environmental exposures (Blauwendraat et al. 2020). Despite its sporadic nature of occurrence, the etiology of sporadic PD is now considered multifactorial, with contributing factors including genetic susceptibility and environmental influences such as pesticide or heavy metal exposure (Chai and Lim 2013). On the other hand, studies of familial PD—through genetic history analysis and genome-wide association studies (GWAS)—have identified several highly penetrant variants associated with disease onset. While certain single-gene mutations appear to be causally linked to familial PD, it is likely that additional genetic modifiers or environmental factors also influence disease manifestation and progression. Some of the identified variants include *SNCA* (*PARK1*, encoding α-syn) (Polymeropoulos et al. 1997), *Parkin* (*PARK7*, encoding PRKN) (Kitada et al. 1998; Leroy et al. 1998), *leucine-rich repeat kinase 2* (*LRRK2*) (Cookson 2010), and *PTEN Induced Kinase 1* (*PINK1*) (Valente et al. 2004). The underlying molecular mechanisms of these disease-causing variants have been studied extensively. For instance, PRKN and PINK1 have been implicated in mitochondrial and mitophagy function. However, it remains unclear whether these mechanisms also contribute to the pathogenesis of sporadic PD.

Among all variants, GWAS have further reinforced the role of α-syn in PD, with findings such as *SNCA* gene triplication linked to familial PD (Singleton et al. 2003), and numerous single nucleotide polymorphisms within *SNCA* associated with increased disease risk (Simón-Sánchez et al. 2009). α-syn is a 140 amino-acid protein composed of three domains: a positively charged N-terminal region, a central hydrophobic region containing the nonamyloid-β component (NAC), and an acidic C-terminal region (Lashuel et al. 2013). Under physiological conditions, monomeric α-syn is predominantly expressed at presynaptic terminals, where it modulates soluble N-ethylmaleimide-sensitive factor activating protein receptor complex assembly and vesicular neurotransmitter release (Maroteaux et al. 1988; Lashuel et al. 2013). In addition to monomers, α-syn α-helical tetramers have also been described (Bartels et al. 2011). These physiological species are generally resistant to fibrillization. However, under pathological conditions, monomeric α-syn undergoes misfolding to form soluble oligomers and insoluble fibrils, eventually aggregating into LBs. Several factors influence α-syn aggregation: (1) the central hydrophobic NAC domain is key to fibril formation, with increased α-syn concentration promoting aggregation (Waxman et al. 2009); (2) familial PD-linked mutations in *SNCA*, such as A30P and A53T, alter fibrillization efficiency (Narhi et al. 1999; Li et al. 2001; Jo et al. 2002; Polymeropoulos et al. 1997; Krüger et al. 1998); and (3) post-translational modifications such as phosphorylation, ubiquitination, and nitration modulate α-syn solubility and aggregation propensity (Oueslati et al. 2010; Fujiwara et al. 2002). Among these, phosphorylation at serine 129 of α-syn is most prominently associated with pathology, as it is the dominant α-syn species found in LBs (Fujiwara et al. 2002).

Intriguingly, the widespread occurrence of α-syn inclusions is observed in not only neurons but also glia, highlighting the involvement of multiple cell types in PD pathogenesis (Arai et al. 1999; Wakabayashi et al. 2000). Insights into how these α-syn inclusions are formed and eliminated in different cell types are subjects of rising interest and of great therapeutic potential. Here we review our current knowledge of different clearance pathways for α-syn in both neurons and glia. Literature published within the last 5 years and indexed by PubMed was thoroughly reviewed. We begin by discussing α-syn-based PD models that have been instrumental in probing these pathways, followed by an overview of cell type-specific degradation systems. While the functional implications of α-syn in disease have been extensively discussed elsewhere (Brás et al. 2020; Peng et al. 2020; Yi et al. 2022), we focus here on the mechanisms governing α-syn clearance and their relevance to therapeutic strategies.

2 | PD Animal Models

Investigations into the mechanisms responsible for α-syn clearance have largely relied on various experimental models that recapitulate key pathological features of PD. Among the most widely used are neurotoxin-based models and transgenic animal models expressing wild-type or mutant forms of human α-syn. Neurotoxin models—such as those utilizing 6-hydroxydopamine, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, or rotenone (a mitochondrial complex I inhibitor)—induce selective degeneration of dopaminergic neurons in *SNpc*, thereby mimicking the hallmark DA neurodegeneration observed in PD patients (Schober 2004; Tieu 2011; Cannon et al. 2009; Van Laar et al. 2023). In addition, motor deficits, α-syn aggregation, and oxidative damage have been detected in animals treated with these neurotoxins, demonstrating that this approach effectively models many key features of PD (McCormack et al. 2008; Rocha et al. 2022; Sanders and Timothy Greenamyre 2013). Although the severity of phenotypes can vary, these models allow rapid and reproducible assessment of motor deficits using behavioral assays including the amphetamine-induced rotation test, rotarod, and pole test—depending on the species and experimental context. The unilateral lesioning approach, in particular, provides a robust internal control, enabling direct comparisons between affected and unaffected hemispheres. Thus, these models are invaluable for high-throughput screening of neuroprotective compounds. Nonetheless, many agents that show efficacy in preclinical neurotoxin models have failed to demonstrate

clinical potential in human trials, raising concerns about their translational relevance (Athauda and Foltynie 2015).

In parallel, transgenic animal models overexpressing either wild-type or familial PD-linked mutant forms of human α-syn have been widely used (Kahle et al. 2000; Masliah et al. 2000; van der Putten et al. 2000). These models typically exhibit robust formation of LB-like inclusions, alterations in striatal dopamine levels, neuroinflammatory responses, and early nonmotor symptoms such as olfactory dysfunction and sleep disturbances (Dovonou et al. 2023; Koprich et al. 2017). Despite clear evidence of α-syn pathology, significant neurodegeneration is often absent or mild, posing challenges for studying the full course of PD progression and for testing disease-modifying therapies targeting neuroprotection.

While traditional neurotoxin and transgenic models offer valuable insights into distinct aspects of PD pathophysiology and α-syn biology, they fall short of fully replicating the multifaceted nature of the disease. These limitations underscore the need for complementary models that better recapitulate both the molecular mechanisms and the progressive neurodegeneration seen in human PD. Recent advances in induced pluripotent stem cell technology have enabled the modeling of PD using patient-derived DA neurons and brain organoids (Bose et al. 2022). These models recapitulate several disease-related phenotypes and offer valuable insights into PD mechanisms. However, their partial in vitro nature limits how accurately they reflect the complex environment in pathological brains. Below, we discuss three widely used in vivo PD models that complement these systems and offer strong potential for advancing our understanding of disease pathogenesis.

2.1 | α-Syn Preformed Fibril (PFF) Injection

To model PD disease progression, exogenous PFF injection generated from recombinant monomeric α-syn proteins has been added to primary neuronal cultures or brains to monitor the formation of α-syn inclusions. Interestingly, the resulting inclusions share several key characteristics with LBs and LNs: they are filamentous by electron microscopy, threadlike, and spherical (Luk et al. 2012; Volpicelli-Daley et al. 2014; Volpicelli-Daley et al. 2011). These sonicated fibrils serve as seeds, triggering endogenous α-syn to be hyperphosphorylated at Ser129 and ubiquitinated for the formation of detergent-resistant aggregates. This conversion of normal endogenous α-syn into the pathological conformation grows and propagates in vivo, accompanying the occurrence of DA neurodegeneration and motor deficits. The mechanism of converting endogenous α-syn to pathological inclusions is unclear. However, the pathological inclusions and subsequent pathology fail to occur in α-syn knockout mice, suggesting that the presence of endogenous α-syn is required for the pathological aggregation and propagation (Luk et al. 2012; Volpicelli-Daley et al. 2014; Volpicelli-Daley et al. 2011).

The PFF model offers several key advantages over other PD models, making it a valuable tool for studying α-syn pathology (Chung et al. 2019; Thakur et al. 2017; Duffy et al. 2018). First, since aggregation and propagation are seeded by exogenous fibrils but driven by endogenous α-syn, the pathological process unfolds under more physiological conditions—unlike viral or transgenic models where α-syn is overexpressed at non-native levels. Second, α-syn inclusions in the PFF model initially appear in axons and later spread to the soma and dendrites, closely mirroring the progression pattern described in the Braak staging hypothesis. Third, the timeline of PFF-induced DA neurodegeneration precedes the onset of motor symptoms, reflecting the prodromal progression observed in human PD. Furthermore, the PFF model is highly versatile in terms of the types of injected fibrils (mouse vs. human α-syn, wild-type vs. mutant, oligomeric vs. fibrillar forms), the stereotaxic coordinates (unilateral or bilateral), and the brain regions targeted. This flexibility allows fine-tuned manipulation of pathology in both spatial and temporal manners. Collectively, the PFF model has proven instrumental in uncovering key aspects of α-syn aggregation, transmission, and the progressive nature of PD pathology.

2.2 | Adeno-Associated Virus (AAV)-Mediated α-Syn Viral Expression

Unilateral AAV injection of human α-syn into the rodent *SNpc* models several key aspects of PD pathology, including neurochemical changes (early and late fluctuations in striatal dopamine), motor dysfunction, and neuroinflammatory changes in the brains. Notably, a progressive loss of DA neurons in the *SNpc* and the tyrosine hydroxylase-positive terminals in the striatum is detected, although the extent of degeneration varies. Initial studies in the early 2000s demonstrated that AAV2 achieves robust expression of human α-syn in the rat *SNpc* DA neurons (Kirik et al. 2002; Yamada et al. 2004; Björklund and Mattsson 2024; Huntington and Srinivasan 2021; Volpicelli-Daley et al. 2016). Later, other serotypes such as AAV2/1, 2/5, 2/6, 2/7, 2/8, and 2/9 were used to deliver wild-type or mutant (A53T, A30P) α-syn in rats and C57BL/6 mice. While enhanced transduction efficiency was achieved, the severity of DA neuron loss varied. Regardless of the serotype, overexpression of either wild-type or mutant α-syn led to progressive neurodegeneration, loss of striatal dopamine, and associated behavioral deficits (Oliveras-Salvá et al. 2013; St Martin et al. 2007; Björklund and Mattsson 2024; Huntington and Srinivasan 2021; Volpicelli-Daley et al. 2016). Furthermore, depending on the dosage, the viral α-syn expression models pre-, early, or advanced symptomatic stages, each exhibiting different degrees of axonopathy (swelling), loss of tyrosine hydroxylase-positive terminals in the striatum, motor impairments, and DA neuron loss (Björklund and Mattsson 2024). In this regard, several factors contribute to the variability in phenotypic outcomes. These include AAV elements such as promoter and enhancer sequences, capsid serotype, purification techniques, α-syn variant or strain used, and the method for determining viral titer (Burger et al. 2004; Hammond et al. 2017; Taymans et al. 2007). As such, batch-to-batch testing of viral efficiency in vivo is essential for reproducibility and interpretation of results (Björklund and Mattsson 2024; Huntington and Srinivasan 2021; Volpicelli-Daley et al. 2016).

2.3 | Fly PD Model

Accumulating evidence supports *Drosophila melanogaster* as a powerful and versatile model organism for studying PD

(Muñoz-Soriano and Paricio 2011; Xiong and Yu 2018; Dabool et al. 2019). Its short life cycle, high fecundity, and ease of genetic manipulation make it especially suitable for large-scale genetic and pharmacological screens. Importantly, despite evolutionary distance from mammals, *Drosophila* possesses conserved DA neuron clusters that are functionally analogous to those in the human brains, enabling meaningful modeling of PD-related neuronal dysfunction.

The utility of *Drosophila* in PD research was first demonstrated in a landmark study in 2000, which showed that pan-neuronal expression of human α-syn leads to selective degeneration of DA neurons, mimicking a hallmark feature of PD pathology (Feany and Bender 2000). In addition to DA neuron loss, these α-syn-expressing flies displayed robust behavioral phenotypes reminiscent of PD, such as impaired climbing ability (a proxy for motor deficits) and reduced lifespan. These early findings provided compelling evidence that *Drosophila* can be used to model both the molecular and behavioral aspects of PD. Subsequently, numerous studies have focused on *Drosophila* homologs of PD-associated genes identified in human patients. Mutations, loss-of-function, or gain-of-function in genes such as *parkin*, *PINK1*, and *LRRK2* in flies recapitulate key PD-like phenotypes, including mitochondrial defects, locomotor impairments, and age-dependent neurodegeneration (Imai 2020; Seegobin et al. 2020). Their interactions with α-syn have also been probed under various genetic conditions (Todd and Staveley 2008; Haywood and Staveley 2006; Todd and Staveley 2012; Narwal et al. 2023). These genetic models have not only helped delineate conserved pathogenic mechanisms underlying PD but also facilitated the identification of potential therapeutic targets. For instance, α-syn modifiers have been identified out of rapid and high-throughput genetic screens using *Drosophila* (Ren et al. 2022). Collectively, these studies firmly establish *Drosophila* as an invaluable in vivo platform for mechanistic dissection and preclinical testing in PD research.

3 | α-Syn Toxicity and Neuronal Death

Numerous studies have established that endoplasmic reticulum (ER) homeostasis plays a critical role in α-syn toxicity, neuronal death, and PD (Ghemrawi and Khair 2020). Abnormal accumulation of α-syn induces ER stress and fragmentation, triggering activation of the unfolded protein response and endoplasmic reticulum-associated degradation, thereby disrupting ER homeostasis. Maintaining ER homeostasis is particularly vital for DA neuron survival, as these neurons are especially vulnerable to cellular equilibrium and damage. Overexpression of α-syn in the ER causes significant loss of DA neurons and motor deficits, recapitulating PD features (Zeng et al. 2024; Colla et al. 2012). Furthermore, enhancing ER proteostasis machinery and trafficking has been shown to improve deficits in ER morphology and lysosomal function, while also reducing α-syn accumulation, underscoring the importance of ER homeostasis in PD pathology (Stojkovska et al. 2022).

α-syn toxicity can also be mitigated through other mechanisms, including selective degradation of ER via ER-phagy, which helps maintain cellular homeostasis. Upon ER stress induction by α-syn accumulation, ER-phagy clears α-syn and restores ER function through interaction with the ER-phagy receptor Family with sequence similarity 134, member B, FAM134B. Overexpression of FAM134B enhances ER-phagy, exerting neuroprotective effects on DA neurons and improving motor performance. These findings suggest that ER-phagy represents a specific ER clearance pathway critical for degrading α-syn (Kim et al. 2023).

Additionally, mice deficient in the nuclear factor erythroid 2-related factor 2 (Nrf2) and overexpressing human α-syn show increased reactive oxygen species (ROS), lipid peroxidation, and free iron accumulation, leading to heightened ferroptosis—a form of iron-dependent regulated cell death. This results in age-dependent α-syn pathology and behavioral deficits reminiscent of PD. It has been proposed that α-syn decreases Nrf2 protein levels, creating a vicious cycle of α-syn overexpression and Nrf2 suppression that promotes neuronal ferroptotic death (Anandhan et al. 2022). Moreover, α-syn is implicated in activating the mitochondrial permeability transition pore (mPTP), a key event in ferroptosis (Ganguly et al. 2024) (Figure 1).

4 | Neuronal Degradation of α-Syn

Despite intensive studies, how α-syn-containing LBs are eliminated and how α-syn proteins are degraded remain largely unclear. In addition to α-syn, LBs harbor ubiquitinated proteins and are often surrounded by autophagosome- and lysosome-like structures, implicating multiple degradation pathways in their clearance (Hoffmann et al. 2019). Thus, like other intracellular proteins, α-syn is likely degraded through the ubiquitin–proteasome system (UPS) (Bi et al. 2021; Lim and Tan 2007) and the autophagy–lysosome pathway (ALP). In 2001, impaired expression of proteasomal subunits and reduced peptidase activities in DA neurons were first reported in PD (McNaught et al. 2003; McNaught and Jenner 2001). Subsequent studies have demonstrated that disrupting proteasomal function by depleting various proteasomal subunits—including Rpt2, Rpt3, Rpn11, or Rpn6—leads to the accumulation of misfolded proteins or Lewy body–like inclusions, accompanied by motor deficits characteristic of PD (Bedford et al. 2008; Pathare et al. 2012; Tashiro et al. 2012; Tonoki et al. 2009; Wahl et al. 2008). Overexpression of the ubiquitin ligase Nedd4, which has been shown to target α-syn for endosomal/lysosomal degradation, reduced α-syn levels and protects against AAV-α-syn-induced neurodegeneration in the *SNpc* (Chung et al. 2013; Davies et al. 2014; Tardiff et al. 2013). Furthermore, pharmacological inhibition of the proteasome with compounds like MG132 in differentiated neuronal cell lines results in enhanced levels of α-syn, suggesting proteasomal degradation of α-syn (Bennett et al. 1999; Webb et al. 2003; Choi et al. 2022). Systemic administration of proteasome inhibitors in rats induces the formation of α-syn and ubiquitin-positive inclusions resembling LBs in surviving neurons, leading to progressive motor deficits that closely mimic the key pathological features of PD (McNaught et al. 2004). On the other hand, numerous studies have shown that α-syn inhibits proteasome activity, particularly when present in oligomeric or fibrillar forms (McKinnon et al. 2020; Tanaka et al. 2001). Proteasomal degradation is disrupted in various cell lines expressing wild-type or mutant α-syn (Chen et al. 2005; Emmanouilidou et al. 2010; Lindersson et al. 2004; Snyder et al. 2003; Tanaka et al. 2001).

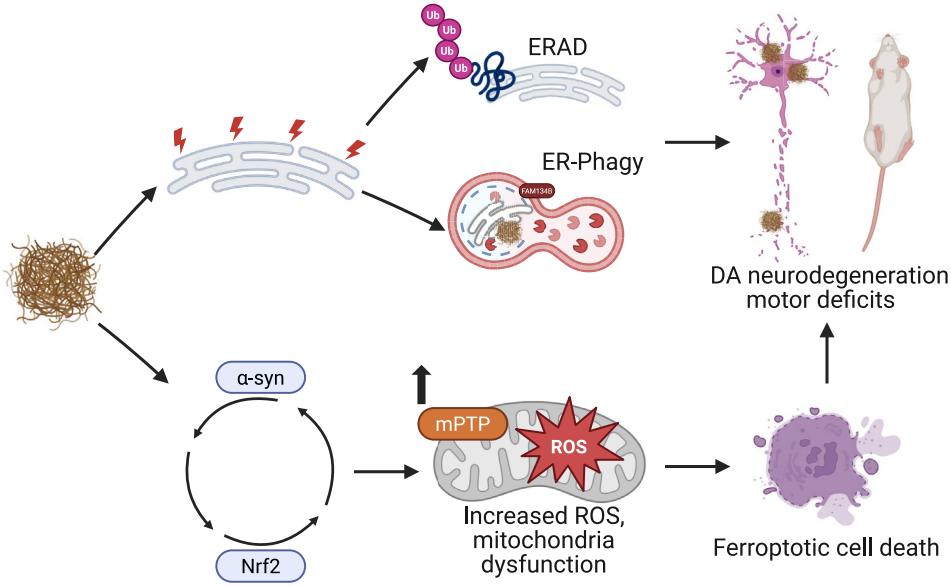

FIGURE 1 | α-syn toxicity and neuronal death. Abnormal accumulation of α-syn induces endoplasmic reticulum (ER) stress, causing a disruption in ER homeostatsis. Under stress conditions, ER-related pathways such as ER-associated degradation (ERAD), ER-Phagy, and unfold protein response (UPR, not shown) are dysfunctional, leading to motor deficits and DA neurodegeneration, features of PD. On the other hand, α-syn accumulation decreases erythroid 2-related factor 2 protein levels, increases the level of reactive oxygen species (ROS), and regulates mitochondrial function via the activation of the mitochondrial permeability transition pore (mPTP). These changes result in ferroptotic cell death and eventually DA neurodegeneration and motor deficits.

Overall, impaired proteasome function exacerbates α-syn accumulation, which further binds to and inhibits the proteasome's proteolytic activity.

Three major ALP pathways have been described: macroautophagy (commonly referred to as autophagy), chaperone-mediated autophagy (CMA), and microautophagy. Among these, CMA is a selective lysosomal degradation pathway that targets short-lived, soluble cytosolic proteins bearing a specific pentapeptide motif recognized by the molecular chaperone heat shock cognate 70 (Dice 2007). Proteins containing this motif—including α-syn—are delivered to lysosomes through interaction with lysosome-associated membrane protein 2A (LAMP2A), which mediates their translocation across the lysosomal membrane. Disruption of the CMA recognition motif in α-syn or genetic downregulation of LAMP2A results in α-syn accumulation in neuronal cell lines and primary neurons, highlighting CMA as a key route for α-syn clearance (Cuervo et al. 2004; Vogiatzi et al. 2008). Supporting this, reduced LAMP2A expression has been reported in early-stage PD patient brains, accompanied by elevated levels of α-syn and other CMA substrates such as myocyte-specific enhancer factor 2D (Murphy et al. 2015; Vogiatzi et al. 2008). Furthermore, knockdown of LAMP2A expression in rodent models leads to the accumulation of ubiquitin-positive α-syn inclusions in the *SNpc*, followed by progressive DA neuron loss (Xilouri et al. 2016). Taken together, these findings underscore the importance of CMA in regulating neuronal α-syn homeostasis.

Pharmacological inhibition of autophagy—such as with 3-methyladenine—in differentiated neuronal cell lines increases α-syn levels, indicating that α-syn is also degraded through autophagy (Vogiatzi et al. 2008; Webb et al. 2003). Autophagy is a tightly regulated degradative process orchestrated by autophagy-related genes (ATGs). Under cellular stress, the ATG1/UNC-51-like kinase 1 (ATG1/ULK1) complex initiates autophagosome formation by recruiting the class III phosphatidylinositol 3-kinase (PI3K) complex, which generates phosphatidylinositol 3-phosphate and drives nucleation of the isolation membrane (phagophore) (Russell et al. 2013). Phagophore expansion is mediated by two ubiquitin-like conjugation systems—ATG12–ATG5 and ATG8 lipidation—and fueled by ATG9-positive vesicles sourced from the Golgi, recycling endosomes, or plasma membrane (Mari et al. 2010; Yamamoto et al. 2012). Upon closure, the double-membrane autophagosome fuses with lysosomes to form autolysosomes, where hydrolases degrade enclosed cargos (Mizushima 2020; Galluzzi et al. 2017; Klionsky et al. 2016).

It has been shown that autophagy function in neurons is crucial for α-syn degradation in vivo. Conditional deletion of *Atg7* in DA neurons leads to the accumulation of ubiquitinated inclusions and age-dependent neurodegeneration (Hunn et al. 2019; Sato et al. 2018; Friedman et al. 2012). Conversely, upregulation of autophagy through overexpression of *Atg7* or *Beclin-1* lowers α-syn levels and ameliorates neurodegeneration in α-syn-overexpressing transgenic mice (Spencer et al. 2009). These findings underscore the importance of autophagy, alongside CMA, in α-syn degradation. It is noteworthy that these degradation pathways may compensate for each other. For instance, autophagy deficiency in neurons does not necessarily result in an overall increase in brain α-syn levels, possibly due to the compensatory action from other systems (Hunn et al. 2019). Based on a plethora of evidence in the past decades, it is in consensus that endogenous α-syn, like monomeric or prefibrillar α-syn, is targeted by UPS (Bennett et al. 1999; Shimura et al. 2001; Tofaris et al. 2011), whereas ALP is induced under stress conditions and is responsible for the degradation of aggregated and fibrillar α-syn (Ebrahimi-Fakhari et al. 2011) (Figure 2A).

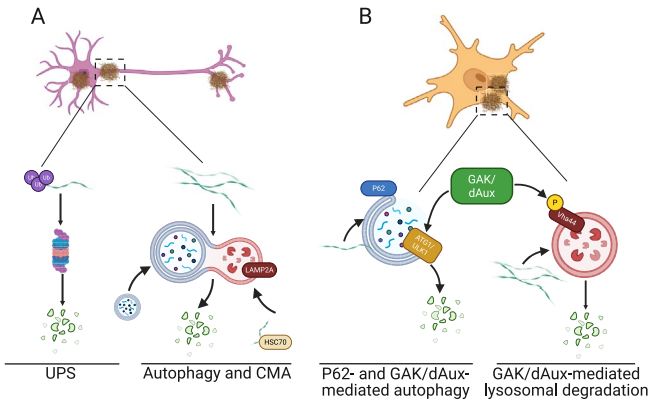

FIGURE 2 | α-syn degradation in neurons and glia in PD. (A) monomeric or prefibrillar α-syn are eliminated in neurons via the UPS pathway. Under pathological conditions, fibrillar α-syn are targeted by autophagy while CMA is the major route for monomeric or prefibrillar α-syn. (B) In activated microglia, α-syn fibrils are targeted by P62-mediated selective autophagy. On the other hand, α-syn is degraded via GAK/dAux-mediated autophagy mechanisms in adult fly glia. GAK/dAux also controls lysosomal acidification via phosphorylation of Vha44, the V_1 C subunit of V-ATPase, for degrading α-syn in adult fly glia.

5 | Glial Degradation of α-Syn: Microglia and Adult Fly Glia

Given that α-syn inclusions are observed in both neurons and glia, increasing attention has focused on whether glial cells, particularly microglia, employ similar degradation mechanisms to clear α-syn. Emerging evidence suggests that autophagy is essential in microglial responses in PD pathology (Gao et al. 2023; Cheng et al. 2020; Qin et al. 2021; Zhang et al. 2025; Zhang et al. 2023). Conditional deletion of Atg7 in microglia leads to behavioral phenotypes resembling autism spectrum disorder, along with impaired synaptosome degradation, altered spine density, and disrupted brain connectivity (Kim et al. 2017). Additionally, deficiency in microglial autophagy has been linked to increased oligodendrocyte numbers and heightened seizure susceptibility, indicating non–cell-autonomous effects (Alam et al. 2021). In models of Alzheimer's disease, loss of microglial Atg7 results in defective Aβ clearance and increased tau pathology (Cho et al. 2014; Xu et al. 2021). Together, these findings highlight the vital role of microglial autophagy in maintaining brain homeostasis and clearing pathological aggregates in neurodegenerative diseases.

Recent studies have uncovered a pivotal role for microglial autophagy in the clearance of α-syn. Choi et al. demonstrated that microglia internalize neuron-released α-syn and degrade it through p62-mediated selective autophagy (Choi, Seegobin, et al. 2020; Choi, Zhang, et al. 2020). In both acute (AAV-α-syn injection) and chronic (Thy1-human α-syn transgenic mice) PD models, microglia-specific deletion of Atg7 exacerbated α-syn inclusions, increased S129 phosphorylated α-syn, and worsened DA neurodegeneration. Interestingly, two recent studies also demonstrated the importance of glial ALP in α-syn clearance. An initial behavioral screen monitoring fly climbing distance first identified dAuxilin (dAux), the fly homolog of the PD risk factor Cyclin-G-associated kinase (GAK), as a key modulator for a broad spectrum of PD-like symptoms in flies (Song et al. 2017). Using both fly and mouse PD models, subsequent work showed that GAK/dAux regulates autophagy initiation in adult fly glia and mouse microglia (Zhang et al. 2023). Loss of GAK/dAux leads to increased size and number of autophagosomes and upregulation of autophagy-related components, including members of the ATG1/ULK1 and class III PI3K complexes. Moreover, GAK/dAux interacts with ATG1/ULK1 via its uncoating domain and regulates the trafficking of Atg1 and Atg9 to autophagosomes, controlling the onset of autophagy in glia. Importantly, GAK/dAux deficiency contributes to PD-like phenotypes in flies, including progressive DA neuron loss and locomotion dysfunction (Wang et al. 2024; Zhang et al. 2023). Additionally, GAK/dAux was shown to regulate phosphorylation at two key Atg9 sites, reinforcing its role in glial autophagy regulation (Wang et al. 2025; Yi et al. 2024).

Intriguingly, GAK/dAux plays additional roles in controlling glial α-syn clearance (Zhang et al. 2025). Lack of glial GAK/dAux increases the lysosome number and size, impairs lysosomal acidification, and reduces hydrolase activity—ultimately blocking the degradation of substrates including α-syn. In adult fly glia lacking dAux, α-syn accumulates predominantly within dysfunctional lysosomes, and exogenous α-syn PFF levels are further elevated in the absence of microglial GAK. Mechanistically, dAux facilitates the phosphorylation at the serine 543 of Vha44, the V_1 C subunit of the vacuolar-type H^+-translocating ATPase, and promotes proper assembly. This phosphorylation-dependent control of lysosomal acidification is essential for efficient α-syn degradation. Finally, disruption in these glial lysosome-dependent mechanisms causes defective α-syn clearance, locomotor deficits, and DA neurodegeneration, linking the mechanistic basis with PD pathology. Together with findings from other studies, these results highlight the importance of glial ALP in eliminating pathological aggregates, offering new insights into therapeutic targets for slowing disease progression in PD (Quick et al. 2023; Yi et al. 2022) (Figure 2B).

6 | Concluding Remarks

The clearance of α-syn is a central determinant in the pathogenesis and progression of PD. A growing body of evidence highlights the multifaceted nature of α-syn homeostasis, including the coordination of intracellular degradation systems such as UPS and ALP in both neurons and glia. These pathways manage misfolded and aggregated α-syn, hence influencing their transcellular propagation, ultimately shaping the spatial–temporal trajectory of neurodegeneration in the brains. Glial cells—particularly astrocytes and microglia—are emerging as key players in the uptake, degradation, and inflammatory modulation of α-syn pathology. Their ability to internalize and process toxic aggregates suggests that therapeutic strategies aimed at enhancing glial clearance mechanisms may offer neuroprotection and potentially slow disease progression. Despite significant advances, many questions remain regarding the efficiency, regulation, and pathological alterations of these clearance systems in the diseased brains. Thus, a deeper understanding of the molecular and cellular pathways governing α-syn clearance will be instrumental in shaping next-generation interventions for PD. Targeting these pathways—particularly enhancing glial clearance capacities—offers promising avenues for mitigating α-syn accumulation and

its associated neurodegenerative consequences. Future research should aim to elucidate the regulatory cross-talk between different degradation systems and among cells, identify biomarkers of impaired clearance, and develop disease-modifying strategies to restore or augment these mechanisms for clinical interventions. In addition, exploring additional α-syn clearance mechanisms in systems beyond the central nervous system will be crucial for developing a more comprehensive understanding of its pathobiology and identifying novel therapeutic opportunities.

Author Contributions

Margaret S. Ho: conceptualization, writing – original draft, writing – review and editing, funding acquisition.

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Ethics Statement

The author has nothing to report.

Conflicts of Interest

The author declares no conflicts of interest.

Data Availability Statement

All data in this study are from available published articles.

Peer Review

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References

Aarsland, D., L. Batzu, G. M. Halliday, et al. 2021. "Parkinson Disease-Associated Cognitive Impairment." *Nature Reviews Disease Primers* 7: 47.

Alam, M. M., X. F. Zhao, Y. Liao, et al. 2021. "Deficiency of Microglial Autophagy Increases the Density of Oligodendrocytes and Susceptibility to Severe Forms of Seizures." *ENeuro* 8.

Anandhan, A., W. Chen, N. Nguyen, L. Madhavan, M. Dodson, and D. D. Zhang. 2022. "α-Syn Overexpression, NRF2 Suppression, and Enhanced Ferroptosis Create a Vicious Cycle of Neuronal Loss in Parkinson's Disease." *Free Radical Biology & Medicine* 192: 130–140.

Arai, T., K. Uéda, K. Ikeda, et al. 1999. "Argyrophilic Glial Inclusions in the Midbrain of Patients With Parkinson's Disease and Diffuse Lewy Body Disease Are Immunopositive for NACP/Alpha-Synuclein." *Neuroscience Letters* 259: 83–86.

Athauda, D., and T. Foltynie. 2015. "The Ongoing Pursuit of Neuroprotective Therapies in Parkinson Disease." *Nature Reviews. Neurology* 11: 25–40.

Baba, M., S. Nakajo, P. H. Tu, et al. 1998. "Aggregation of Alpha-Synuclein in Lewy Bodies of Sporadic Parkinson's Disease and Dementia With Lewy Bodies." *American Journal of Pathology* 152: 879–884.

Bartels, T., J. G. Choi, and D. J. Selkoe. 2011. "α-Synuclein Occurs Physiologically as a Helically Folded Tetramer That Resists Aggregation." *Nature* 477: 107–110.

Bedford, L., D. Hay, A. Devoy, et al. 2008. "Depletion of 26S Proteasomes in Mouse Brain Neurons Causes Neurodegeneration and Lewy-Like Inclusions Resembling Human Pale Bodies." *Journal of Neuroscience* 28: 8189–8198.

Bennett, M. C., J. F. Bishop, Y. Leng, P. B. Chock, T. N. Chase, and M. M. Mouradian. 1999. "Degradation of Alpha-Synuclein by Proteasome." *Journal of Biological Chemistry* 274: 33855–33858.

Bi, M., X. Du, Q. Jiao, X. Chen, and H. Jiang. 2021. "Expanding the Role of Proteasome Homeostasis in Parkinson's Disease: Beyond Protein Breakdown." *Cell Death & Disease* 12: 154.

Björklund, A., and B. Mattsson. 2024. "The AAV-α-Synuclein Model of Parkinson's Disease: An Update." *Journal of Parkinson's Disease* 14: 1077–1094.

Blauwendraat, C., M. A. Nalls, and A. B. Singleton. 2020. "The Genetic Architecture of Parkinson's Disease." *Lancet Neurology* 19: 170–178.

Bose, A., G. A. Petsko, and L. Studer. 2022. "Induced Pluripotent Stem Cells: A Tool for Modeling Parkinson's Disease." *Trends in Neurosciences* 45: 608–620.

Braak, H., K. Del Tredici, U. Rüb, R. A. de Vos, E. N. Jansen Steur, and E. Braak. 2003. "Staging of Brain Pathology Related to Sporadic Parkinson's Disease." *Neurobiology of Aging* 24: 197–211.

Brás, I. C., A. Dominguez-Meijide, E. Gerhardt, et al. 2020. "Synucleinopathies: Where We Are and Where We Need to Go." *Journal of Neurochemistry* 153: 433–454.

Burger, C., O. S. Gorbatyuk, M. J. Velardo, et al. 2004. "Recombinant AAV Viral Vectors Pseudotyped With Viral Capsids From Serotypes 1, 2, and 5 Display Differential Efficiency and Cell Tropism After Delivery to Different Regions of the Central Nervous System." *Molecular Therapy* 10: 302–317.

Cannon, J. R., V. Tapias, H. M. Na, A. S. Honick, R. E. Drolet, and J. T. Greenamyre. 2009. "A Highly Reproducible Rotenone Model of Parkinson's Disease." *Neurobiology of Disease* 34: 279–290.

Chai, C., and K. L. Lim. 2013. "Genetic Insights Into Sporadic Parkinson's Disease Pathogenesis." *Current Genomics* 14: 486–501.

Chen, Q., J. Thorpe, and J. N. Keller. 2005. "Alpha-Synuclein Alters Proteasome Function, Protein Synthesis, and Stationary Phase Viability." *Journal of Biological Chemistry* 280: 30009–30017.

Cheng, J., Y. Liao, Y. Dong, et al. 2020. "Microglial Autophagy Defect Causes Parkinson Disease-Like Symptoms by Accelerating Inflammasome Activation in Mice." *Autophagy* 16: 2193–2205.

Cho, M. H., K. Cho, H. J. Kang, et al. 2014. "Autophagy in Microglia Degrades Extracellular β-Amyloid Fibrils and Regulates the NLRP3 Inflammasome." *Autophagy* 10: 1761–1775.

Choi, I., G. R. Heaton, Y. K. Lee, and Z. Yue. 2022. "Regulation of α-Synuclein Homeostasis and Inflammasome Activation by Microglial Autophagy." *Science Advances* 8: eabn1298.

Choi, I., S. P. Seegobin, D. Liang, and Z. Yue. 2020. "Synucleinphagy: A Microglial 'Community Cleanup Program' for Neuroprotection." *Autophagy* 16: 1718–1720.

Choi, I., Y. Zhang, S. P. Seegobin, et al. 2020. "Microglia Clear Neuron-Released α-Synuclein via Selective Autophagy and Prevent Neurodegeneration." *Nature Communications* 11: 1386.

Chung, C. Y., V. Khurana, P. K. Auluck, et al. 2013. "Identification and Rescue of α-Synuclein Toxicity in Parkinson Patient-Derived Neurons." *Science* 342: 983–987.

Chung, H. K., H. A. Ho, D. Pérez-Acuña, and S. J. Lee. 2019. "Modeling α-Synuclein Propagation With Preformed Fibril Injections." *Journal of Movement Disorders* 12: 139–151.

Colla, E., P. H. Jensen, O. Pletnikova, J. C. Troncoso, C. Glabe, and M. K. Lee. 2012. "Accumulation of Toxic α-Synuclein Oligomer Within Endoplasmic Reticulum Occurs in α-Synucleinopathy In Vivo." *Journal of Neuroscience* 32: 3301–3305.

Cookson, M. R. 2010. "The Role of Leucine-Rich Repeat Kinase 2 (LRRK2) in Parkinson's Disease." *Nature Reviews. Neuroscience* 11: 791–797.

Cuervo, A. M., L. Stefanis, R. Fredenburg, P. T. Lansbury, and D. Sulzer. 2004. "Impaired Degradation of Mutant Alpha-Synuclein by Chaperone-Mediated Autophagy." *Science* 305: 1292–1295.

Dabool, L., L. Juravlev, K. Hakim-Mishnaevski, and E. Kurant. 2019. "Modeling Parkinson's Disease in Adult Drosophila." *Journal of Neuroscience Methods* 311: 89–94.

Davies, S. E., P. J. Hallett, T. Moens, et al. 2014. "Enhanced Ubiquitin-Dependent Degradation by Nedd4 Protects Against α-Synuclein Accumulation and Toxicity in Animal Models of Parkinson's Disease." *Neurobiology of Disease* 64: 79–87.

Del Tredici, K., and H. Braak. 2013. "Dysfunction of the Locus Coeruleus-Norepinephrine System and Related Circuitry in Parkinson's Disease-Related Dementia." *Journal of Neurology, Neurosurgery, and Psychiatry* 84: 774–783.

Dice, J. F. 2007. "Chaperone-Mediated Autophagy." *Autophagy* 3: 295–299.

Dovonou, A., C. Bolduc, V. Soto Linan, C. Gora, M. R. Peralta Iii, and M. Lévesque. 2023. "Animal Models of Parkinson's Disease: Bridging the Gap Between Disease Hallmarks and Research Questions." *Translational Neurodegeneration* 12: 36.

Duffy, M. F., T. J. Collier, J. R. Patterson, et al. 2018. "Quality Over Quantity: Advantages of Using Alpha-Synuclein Preformed Fibril Triggered Synucleinopathy to Model Idiopathic Parkinson's Disease." *Frontiers in Neuroscience* 12, no. 2018: 621.

Ebrahimi-Fakhari, D., I. Cantuti-Castelvetri, Z. Fan, et al. 2011. "Distinct Roles In Vivo for the Ubiquitin-Proteasome System and the Autophagy-Lysosomal Pathway in the Degradation of α-Synuclein." *Journal of Neuroscience* 31: 14508–14520.

Emmanouilidou, E., L. Stefanis, and K. Vekrellis. 2010. "Cell-Produced Alpha-Synuclein Oligomers Are Targeted to, and Impair, the 26S Proteasome." *Neurobiology of Aging* 31: 953–968.

Feany, M. B., and W. W. Bender. 2000. "A Drosophila Model of Parkinson's Disease." *Nature* 404: 394–398.

Fleming, S. M., T. Schallert, and M. R. Ciucci. 2012. "Cranial and Related Sensorimotor Impairments in Rodent Models of Parkinson's Disease." *Behavioural Brain Research* 231: 317–322.

Friedman, L. G., M. L. Lachenmayer, J. Wang, et al. 2012. "Disrupted Autophagy Leads to Dopaminergic Axon and Dendrite Degeneration and Promotes Presynaptic Accumulation of α-Synuclein and LRRK2 in the Brain." *Journal of Neuroscience* 32: 7585–7593.

Fujiwara, H., M. Hasegawa, N. Dohmae, et al. 2002. "Alpha-Synuclein Is Phosphorylated in Synucleinopathy Lesions." *Nature Cell Biology* 4: 160–164.

Galluzzi, L., E. H. Baehrecke, A. Ballabio, et al. 2017. "Molecular Definitions of Autophagy and Related Processes." *EMBO Journal* 36: 1811–1836.

Ganguly, U., S. Singh, A. Bir, et al. 2024. "Alpha-Synuclein Interaction With Mitochondria Is the Final Mechanism of Ferroptotic Death Induced by Erastin in SH-SY5Y Cells." *Free Radical Research* 58: 217–228.

Gao, C., J. Jiang, Y. Tan, and S. Chen. 2023. "Microglia in Neurodegenerative Diseases: Mechanism and Potential Therapeutic Targets." *Signal Transduction and Targeted Therapy* 8: 359.

Ghemrawi, R., and M. Khair. 2020. "Endoplasmic Reticulum Stress and Unfolded Protein Response in Neurodegenerative Diseases." *International Journal of Molecular Sciences* 21: 6127.

Giguère, N., S. Burke Nanni, and L. E. Trudeau. 2018. "On Cell Loss and Selective Vulnerability of Neuronal Populations in Parkinson's Disease." *Frontiers in Neurology* 9: 455.

Hammond, S. L., A. N. Leek, E. H. Richman, and R. B. Tjalkens. 2017. "Cellular Selectivity of AAV Serotypes for Gene Delivery in Neurons and Astrocytes by Neonatal Intracerebroventricular Injection." *PLoS One* 12: e0188830.

Haywood, A. F., and B. E. Staveley. 2006. "Mutant Alpha-Synuclein-Induced Degeneration Is Reduced by Parkin in a Fly Model of Parkinson's Disease." *Genome* 49: 505–510.

Hoffmann, A. C., G. Minakaki, S. Menges, et al. 2019. "Extracellular Aggregated Alpha Synuclein Primarily Triggers Lysosomal Dysfunction in Neural Cells Prevented by Trehalose." *Scientific Reports* 9: 544.

Hunn, B. H. M., S. Vingill, S. Threlfell, et al. 2019. "Impairment of Macroautophagy in Dopamine Neurons Has Opposing Effects on Parkinsonian Pathology and Behavior." *Cell Reports* 29: 920–931.e7.

Huntington, T. E., and R. Srinivasan. 2021. "Adeno-Associated Virus Expression of α-Synuclein as a Tool to Model Parkinson's Disease: Current Understanding and Knowledge Gaps." *Aging and Disease* 12: 1120–1137.

Imai, Y. 2020. "PINK1-Parkin Signaling in Parkinson's Disease: Lessons From Drosophila." *Neuroscience Research* 159: 40–46.

Jo, E., N. Fuller, R. P. Rand, P. St George-Hyslop, and P. E. Fraser. 2002. "Defective Membrane Interactions of Familial Parkinson's Disease Mutant A30P Alpha-Synuclein." *Journal of Molecular Biology* 315: 799–807.

Kahle, P. J., M. Neumann, L. Ozmen, et al. 2000. "Subcellular Localization of Wild-Type and Parkinson's Disease-Associated Mutant Alpha -Synuclein in Human and Transgenic Mouse Brain." *Journal of Neuroscience* 20: 6365–6373.

Kim, D. Y., J. Y. Shin, J. E. Lee, et al. 2023. "A Selective ER-Phagy Exerts Neuroprotective Effects via Modulation of α-Synuclein Clearance in Parkinsonian Models." *Proceedings of the National Academy of Sciences of the United States of America* 120: e2221929120.

Kim, H. J., M. H. Cho, W. H. Shim, et al. 2017. "Deficient Autophagy in Microglia Impairs Synaptic Pruning and Causes Social Behavioral Defects." *Molecular Psychiatry* 22: 1576–1584.

Kirik, D., C. Rosenblad, C. Burger, et al. 2002. "Parkinson-Like Neurodegeneration Induced by Targeted Overexpression of Alpha-Synuclein in the Nigrostriatal System." *Journal of Neuroscience* 22: 2780–2791.

Kitada, T., S. Asakawa, N. Hattori, et al. 1998. "Mutations in the Parkin Gene Cause Autosomal Recessive Juvenile Parkinsonism." *Nature* 392: 605–608.

Klionsky, D. J., K. Abdelmohsen, A. Abe, et al. 2016. "Guidelines for the Use and Interpretation of Assays for Monitoring Autophagy (3rd Edition)." *Autophagy* 12: 1–222.

Koprich, J. B., L. V. Kalia, and J. M. Brotchie. 2017. "Animal Models of α-Synucleinopathy for Parkinson Disease Drug Development." *Nature Reviews. Neuroscience* 18: 515–529.

Kouli, A., K. M. Torsney, and W. L. Kuan. 2018. "Parkinson's Disease: Etiology, Neuropathology, and Pathogenesis." In *Parkinson's Disease:*

Pathogenesis and Clinical Aspects, edited by T. B. Stoker and J. C. Greenland. Codon Publications.

Krüger, R., W. Kuhn, T. Müller, et al. 1998. "Ala30Pro Mutation in the Gene Encoding Alpha-Synuclein in Parkinson's Disease." *Nature Genetics* 18: 106–108.

Lashuel, H. A., C. R. Overk, A. Oueslati, and E. Masliah. 2013. "The Many Faces of α-Synuclein: From Structure and Toxicity to Therapeutic Target." *Nature Reviews. Neuroscience* 14: 38–48.

Lee, H. M., and S. B. Koh. 2015. "Many Faces of Parkinson's Disease: Non-Motor Symptoms of Parkinson's Disease." *Journal of Movement Disorders* 8: 92–97.

Leroy, E., D. Anastasopoulos, S. Konitsiotis, C. Lavedan, and M. H. Polymeropoulos. 1998. "Deletions in the Parkin Gene and Genetic Heterogeneity in a Greek Family With Early Onset Parkinson's Disease." *Human Genetics* 103: 424–427.

Lewis, G. N., and W. D. Byblow. 2002. "Altered Sensorimotor Integration in Parkinson's Disease." *Brain* 125: 2089–2099.

Li, J., V. N. Uversky, and A. L. Fink. 2001. "Effect of Familial Parkinson's Disease Point Mutations A30P and A53T on the Structural Properties, Aggregation, and Fibrillation of Human Alpha-Synuclein." *Biochemistry* 40: 11604–11613.

Lim, K.-L., and J. M. M. Tan. 2007. "Role of the Ubiquitin Proteasome System in Parkinson's Disease." *BMC Biochemistry* 8: S13.

Lindersson, E., R. Beedholm, P. Højrup, et al. 2004. "Proteasomal Inhibition by Alpha-Synuclein Filaments and Oligomers." *Journal of Biological Chemistry* 279: 12924–12934.

Luk, K. C., V. Kehm, J. Carroll, et al. 2012. "Pathological α-Synuclein Transmission Initiates Parkinson-Like Neurodegeneration in Nontransgenic Mice." *Science* 338: 949–953.

Mari, M., J. Griffith, E. Rieter, L. Krishnappa, D. J. Klionsky, and F. Reggiori. 2010. "An Atg9-Containing Compartment That Functions in the Early Steps of Autophagosome Biogenesis." *Journal of Cell Biology* 190: 1005–1022.

Maroteaux, L., J. T. Campanelli, and R. H. Scheller. 1988. "Synuclein: A Neuron-Specific Protein Localized to the Nucleus and Presynaptic Nerve Terminal." *Journal of Neuroscience* 8: 2804–2815.

Masliah, E., E. Rockenstein, I. Veinbergs, et al. 2000. "Dopaminergic Loss and Inclusion Body Formation in Alpha-Synuclein Mice: Implications for Neurodegenerative Disorders." *Science* 287: 1265–1269.

McCormack, A. L., S. K. Mak, M. Shenasa, W. J. Langston, L. S. Forno, and D. A. Di Monte. 2008. "Pathologic Modifications of α-Synuclein in 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine (MPTP)-Treated Squirrel Monkeys." *Journal of Neuropathology & Experimental Neurology* 67: 793–802.

McKinnon, C., M. L. De Snoo, E. Gondard, et al. 2020. "Early-Onset Impairment of the Ubiquitin-Proteasome System in Dopaminergic Neurons Caused by α-Synuclein." *Acta Neuropathologica Communications* 8: 17.

McNaught, K. S., R. Belizaire, O. Isacson, P. Jenner, and C. W. Olanow. 2003. "Altered Proteasomal Function in Sporadic Parkinson's Disease." *Experimental Neurology* 179: 38–46.

McNaught, K. S., and P. Jenner. 2001. "Proteasomal Function Is Impaired in Substantia Nigra in Parkinson's Disease." *Neuroscience Letters* 297: 191–194.

McNaught, K. S., D. P. Perl, A. L. Brownell, and C. W. Olanow. 2004. "Systemic Exposure to Proteasome Inhibitors Causes a Progressive Model of Parkinson's Disease." *Annals of Neurology* 56: 149–162.

Mizushima, N. 2020. "The ATG Conjugation Systems in Autophagy." *Current Opinion in Cell Biology* 63: 1–10.

Müller, M. L., and N. I. Bohnen. 2013. "Cholinergic Dysfunction in Parkinson's Disease." *Current Neurology and Neuroscience Reports* 13: 377.

Muñoz-Soriano, V., and N. Paricio. 2011. "Drosophila Models of Parkinson's Disease: Discovering Relevant Pathways and Novel Therapeutic Strategies." *Parkinson's Disease* 2011: 520640.

Murphy, K. E., A. M. Gysbers, S. K. Abbott, et al. 2015. "Lysosomal-Associated Membrane Protein 2 Isoforms Are Differentially Affected in Early Parkinson's Disease." *Movement Disorders* 30: 1639–1647.

Narhi, L., S. J. Wood, S. Steavenson, et al. 1999. "Both Familial Parkinson's Disease Mutations Accelerate Alpha-Synuclein Aggregation." *Journal of Biological Chemistry* 274: 9843–9846.

Narwal, S., A. Singh, and M. Tare. 2023. "Analysis of α-Syn and Parkin Interaction in Mediating Neuronal Death in Drosophila Model of Parkinson's Disease." *Frontiers in Cellular Neuroscience* 17: 1295805.

Oliveras-Salvá, M., A. Van der Perren, N. Casadei, et al. 2013. "rAAV2/7 Vector-Mediated Overexpression of Alpha-Synuclein in Mouse Substantia Nigra Induces Protein Aggregation and Progressive Dose-Dependent Neurodegeneration." *Molecular Neurodegeneration* 8: 44.

Oueslati, A., M. Fournier, and H. A. Lashuel. 2010. "Role of Post-Translational Modifications in Modulating the Structure, Function and Toxicity of Alpha-Synuclein: Implications for Parkinson's Disease Pathogenesis and Therapies." *Progress in Brain Research* 183: 115–145.

Parkinson, J. 2002. "An Essay on the Shaking Palsy. 1817." *Journal of Neuropsychiatry and Clinical Neurosciences* 14: 223–236; discussion 222.

Pathare, G. R., I. Nagy, S. Bohn, et al. 2012. "The Proteasomal Subunit Rpn6 Is a Molecular Clamp Holding the Core and Regulatory Subcomplexes Together." *Proceedings of the National Academy of Sciences of the United States of America* 109: 149–154.

Peng, C., J. Q. Trojanowski, and V. M. Lee. 2020. "Protein Transmission in Neurodegenerative Disease." *Nature Reviews. Neurology* 16: 199–212.

Perez-Lloret, S., and F. J. Barrantes. 2016. "Deficits in Cholinergic Neurotransmission and Their Clinical Correlates in Parkinson's Disease." *NPJ Parkinson's Disease* 2, no. 16001.

Pfeiffer, R. F. 2016. "Non-Motor Symptoms in Parkinson's Disease." *Parkinsonism & Related Disorders* 22: S119–S122.

Polymeropoulos, M. H., C. Lavedan, E. Leroy, et al. 1997. "Mutation in the Alpha-Synuclein Gene Identified in Families With Parkinson's Disease." *Science* 276: 2045–2047.

Prell, T. 2018. "Structural and Functional Brain Patterns of Non-Motor Syndromes in Parkinson's Disease." *Frontiers in Neurology* 9: 138.

Qin, Y., J. Qiu, P. Wang, et al. 2021. "Impaired Autophagy in Microglia Aggravates Dopaminergic Neurodegeneration by Regulating NLRP3 Inflammasome Activation in Experimental Models of Parkinson's Disease." *Brain, Behavior, and Immunity* 91: 324–338.

Quick, J. D., C. Silva, J. H. Wong, et al. 2023. "Lysosomal Acidification Dysfunction in Microglia: An Emerging Pathogenic Mechanism of Neuroinflammation and Neurodegeneration." *Journal of Neuroinflammation* 20: 185.

Ren, M., Y. Yang, K. H. Y. Heng, et al. 2022. "MED13 and Glycolysis Are Conserved Modifiers of α-Synuclein-Associated Neurodegeneration." *Cell Reports* 41: 111852.

Rocha, S. M., C. M. Bantle, T. Aboellail, D. Chatterjee, R. J. Smeyne, and R. B. Tjalkens. 2022. "Rotenone Induces Regionally Distinct α-Synuclein Protein Aggregation and Activation of Glia Prior to Loss of Dopaminergic Neurons in C57Bl/6 Mice." *Neurobiology of Disease* 167: 105685.

Russell, R. C., Y. Tian, H. Yuan, et al. 2013. "ULK1 Induces Autophagy by Phosphorylating Beclin-1 and Activating VPS34 Lipid Kinase." *Nature Cell Biology* 15: 741–750.

Sanders, L. H., and J. Timothy Greenamyre. 2013. "Oxidative Damage to Macromolecules in Human Parkinson Disease and the Rotenone Model." *Free Radical Biology and Medicine* 62: 111–120.

Sato, S., T. Uchihara, T. Fukuda, et al. 2018. "Loss of Autophagy in Dopaminergic Neurons Causes Lewy Pathology and Motor Dysfunction in Aged Mice." *Scientific Reports* 8: 2813.

Schober, A. 2004. "Classic Toxin-Induced Animal Models of Parkinson's Disease: 6-OHDA and MPTP." *Cell and Tissue Research* 318: 215–224.

Seegobin, S. P., G. R. Heaton, D. Liang, et al. 2020. "Progress in LRRK2-Associated Parkinson's Disease Animal Models." *Frontiers in Neuroscience* 14: 674.

Shimura, H., M. G. Schlossmacher, N. Hattori, et al. 2001. "Ubiquitination of a New Form of Alpha-Synuclein by Parkin From Human Brain: Implications for Parkinson's Disease." *Science* 293: 263–269.

Simón-Sánchez, J., C. Schulte, J. M. Bras, et al. 2009. "Genome-Wide Association Study Reveals Genetic Risk Underlying Parkinson's Disease." *Nature Genetics* 41: 1308–1312.

Singleton, A. B., M. Farrer, J. Johnson, et al. 2003. "Alpha-Synuclein Locus Triplication Causes Parkinson's Disease." *Science* 302: 841.

Snyder, H., K. Mensah, C. Theisler, J. Lee, A. Matouschek, and B. Wolozin. 2003. "Aggregated and Monomeric Alpha-Synuclein Bind to the S6' Proteasomal Protein and Inhibit Proteasomal Function." *Journal of Biological Chemistry* 278: 11753–11759.

Song, L., Y. He, J. Ou, et al. 2017. "Auxilin Underlies Progressive Locomotor Deficits and Dopaminergic Neuron Loss in a Drosophila Model of Parkinson's Disease." *Cell Reports* 18: 1132–1143.

Spencer, B., R. Potkar, M. Trejo, et al. 2009. "Beclin 1 Gene Transfer Activates Autophagy and Ameliorates the Neurodegenerative Pathology in Alpha-Synuclein Models of Parkinson's and Lewy Body Diseases." *Journal of Neuroscience* 29: 13578–13588.

Spillantini, M. G., R. A. Crowther, R. Jakes, M. Hasegawa, and M. Goedert. 1998. "Alpha-Synuclein in Filamentous Inclusions of Lewy Bodies From Parkinson's Disease and Dementia With Lewy Bodies." *Proceedings of the National Academy of Sciences of the United States of America* 95: 6469–6473.

Spillantini, M. G., M. L. Schmidt, V. M. Lee, J. Q. Trojanowski, R. Jakes, and M. Goedert. 1997. "Alpha-Synuclein in Lewy Bodies." *Nature* 388: 839–840.

St Martin, J. L., J. Klucken, T. F. Outeiro, et al. 2007. "Dopaminergic Neuron Loss and Up-Regulation of Chaperone Protein mRNA Induced by Targeted Over-Expression of Alpha-Synuclein in Mouse Substantia Nigra." *Journal of Neurochemistry* 100: 1449–1457.

Stojkovska, I., W. Y. Wani, F. Zunke, et al. 2022. "Rescue of α-Synuclein Aggregation in Parkinson's Patient Neurons by Synergistic Enhancement of ER Proteostasis and Protein Trafficking." *Neuron* 110: 436–451.e11.

Sun, J., J. Ma, L. Gao, et al. 2023. "Disruption of Locus Coeruleus-Related Functional Networks in Parkinson's Disease." *NPJ Parkinson's Disease* 9: 81.

Tanaka, Y., S. Engelender, S. Igarashi, et al. 2001. "Inducible Expression of Mutant Alpha-Synuclein Decreases Proteasome Activity and Increases Sensitivity to Mitochondria-Dependent Apoptosis." *Human Molecular Genetics* 10: 919–926.

Tardiff, D. F., N. T. Jui, V. Khurana, et al. 2013. "Yeast Reveal a 'Druggable' Rsp5/Nedd4 Network That Ameliorates α-Synuclein Toxicity in Neurons." *Science (New York, N.Y.)* 342: 979–983.

Tashiro, Y., M. Urushitani, H. Inoue, et al. 2012. "Motor Neuron-Specific Disruption of Proteasomes, but Not Autophagy, Replicates Amyotrophic Lateral Sclerosis." *Journal of Biological Chemistry* 287: 42984–42994.

Taymans, J. M., L. H. Vandenberghe, C. V. Haute, et al. 2007. "Comparative Analysis of Adeno-Associated Viral Vector Serotypes 1, 2, 5, 7, and 8 in Mouse Brain." *Human Gene Therapy* 18: 195–206.

Thakur, P., L. S. Breger, M. Lundblad, et al. 2017. "Modeling Parkinson's Disease Pathology by Combination of Fibril Seeds and α-Synuclein Overexpression in the Rat Brain." *Proceedings of the National Academy of Sciences of the United States of America* 114: E8284–E8293.

Tieu, K. 2011. "A Guide to Neurotoxic Animal Models of Parkinson's Disease." *Cold Spring Harbor Perspectives in Medicine* 1: a009316.

Todd, A. M., and B. E. Staveley. 2008. "Pink1 Suppresses Alpha-Synuclein-Induced Phenotypes in a Drosophila Model of Parkinson's Disease." *Genome* 51: 1040–1046.

Todd, A. M., and B. E. Staveley. 2012. "Expression of Pink1 With α-Synuclein in the Dopaminergic Neurons of Drosophila Leads to Increases in Both Lifespan and Healthspan." *Genetics and Molecular Research* 11: 1497–1502.

Tofaris, G. K., H. T. Kim, R. Hourez, J. W. Jung, K. P. Kim, and A. L. Goldberg. 2011. "Ubiquitin Ligase Nedd4 Promotes Alpha-Synuclein Degradation by the Endosomal-Lysosomal Pathway." *Proceedings of the National Academy of Sciences of the United States of America* 108: 17004–17009.

Tonoki, A., E. Kuranaga, T. Tomioka, et al. 2009. "Genetic Evidence Linking Age-Dependent Attenuation of the 26S Proteasome With the Aging Process." *Molecular and Cellular Biology* 29: 1095–1106.

Valente, E. M., P. M. Abou-Sleiman, V. Caputo, et al. 2004. "Hereditary Early-Onset Parkinson's Disease Caused by Mutations in PINK1." *Science* 304: 1158–1160.

van der Putten, H., K. H. Wiederhold, A. Probst, et al. 2000. "Neuropathology in Mice Expressing Human Alpha-Synuclein." *Journal of Neuroscience* 20: 6021–6029.

Van Laar, A. D., K. R. Webb, M. T. Keeney, et al. 2023. "Transient Exposure to Rotenone Causes Degeneration and Progressive Parkinsonian Motor Deficits, Neuroinflammation, and Synucleinopathy." *NPJ Parkinson's Disease* 9: 121.

Vogiatzi, T., M. Xilouri, K. Vekrellis, and L. Stefanis. 2008. "Wild Type Alpha-Synuclein Is Degraded by Chaperone-Mediated Autophagy and Macroautophagy in Neuronal Cells." *Journal of Biological Chemistry* 283: 23542–23556.

Volpicelli-Daley, L. A., D. Kirik, L. E. Stoyka, D. G. Standaert, and A. S. Harms. 2016. "How Can rAAV-α-Synuclein and the Fibril α-Synuclein Models Advance Our Understanding of Parkinson's Disease?" *Journal of Neurochemistry* 139, no. Suppl 1: 131–155.

Volpicelli-Daley, L. A., K. C. Luk, and V. M. Lee. 2014. "Addition of Exogenous α-Synuclein Preformed Fibrils to Primary Neuronal Cultures to Seed Recruitment of Endogenous α-Synuclein to Lewy Body and Lewy Neurite-Like Aggregates." *Nature Protocols* 9: 2135–2146.

Volpicelli-Daley, L. A., K. C. Luk, T. P. Patel, et al. 2011. "Exogenous α-Synuclein Fibrils Induce Lewy Body Pathology Leading to Synaptic Dysfunction and Neuron Death." *Neuron* 72: 57–71.

Wahl, C., S. Kautzmann, G. Krebiehl, et al. 2008. "A Comprehensive Genetic Study of the Proteasomal Subunit S6 ATPase in German Parkinson's Disease Patients." *Journal of Neural Transmission (Vienna)* 115: 1141–1148.

Wakabayashi, K., S. Hayashi, M. Yoshimoto, H. Kudo, and H. Takahashi. 2000. "NACP/Alpha-Synuclein-Positive Filamentous Inclusions in

Astrocytes and Oligodendrocytes of Parkinson's Disease Brains." *Acta Neuropathologica* 99: 14–20.

Wang, L., S. Yi, S. Zhang, et al. 2025. "New Atg9 Phosphorylation Sites Regulate Autophagic Trafficking in Glia." *ASN Neuro* 17: 2443442.

Wang, L., S. Zhang, S. Yi, and M. S. Ho. 2024. "A New Regulator of Autophagy Initiation in Glia." *Autophagy* 20: 207–209.

Waxman, E. A., J. R. Mazzulli, and B. I. Giasson. 2009. "Characterization of Hydrophobic Residue Requirements for Alpha-Synuclein Fibrillization." *Biochemistry* 48: 9427–9436.

Webb, J. L., B. Ravikumar, J. Atkins, J. N. Skepper, and D. C. Rubinsztein. 2003. "Alpha-Synuclein Is Degraded by Both Autophagy and the Proteasome." *Journal of Biological Chemistry* 278: 25009–25013.

Xilouri, M., O. R. Brekk, A. Polissidis, M. Chrysanthou-Piterou, I. Kloukina, and L. Stefanis. 2016. "Impairment of Chaperone-Mediated Autophagy Induces Dopaminergic Neurodegeneration in Rats." *Autophagy* 12: 2230–2247.

Xiong, Y., and J. Yu. 2018. "Modeling Parkinson's Disease in Drosophila: What Have we Learned for Dominant Traits?" *Frontiers in Neurology* 9: 228.

Xu, Y., N. E. Propson, S. Du, W. Xiong, and H. Zheng. 2021. "Autophagy Deficiency Modulates Microglial Lipid Homeostasis and Aggravates Tau Pathology and Spreading." *Proceedings of the National Academy of Sciences of the United States of America* 118: e2023418118.

Yamada, M., T. Iwatsubo, Y. Mizuno, and H. Mochizuki. 2004. "Overexpression of Alpha-Synuclein in Rat Substantia Nigra Results in Loss of Dopaminergic Neurons, Phosphorylation of Alpha-Synuclein and Activation of Caspase-9: Resemblance to Pathogenetic Changes in Parkinson's Disease." *Journal of Neurochemistry* 91: 451–461.

Yamamoto, H., S. Kakuta, T. M. Watanabe, et al. 2012. "Atg9 Vesicles Are an Important Membrane Source During Early Steps of Autophagosome Formation." *Journal of Cell Biology* 198: 219–233.

Yi, S., L. Wang, M. S. Ho, and S. Zhang. 2024. "The Autophagy Protein Atg9 Functions in Glia and Contributes to Parkinsonian Symptoms in a Drosophila Model of Parkinson's Disease." *Neural Regeneration Research* 19: 1150–1155.

Yi, S., L. Wang, H. Wang, M. S. Ho, and S. Zhang. 2022. "Pathogenesis of α-Synuclein in Parkinson's Disease: From a Neuron-Glia Crosstalk Perspective." *International Journal of Molecular Sciences* 23: 14753.

Zeng, H., Y. Liu, X. Liu, et al. 2024. "Interplay of α-Synuclein Oligomers and Endoplasmic Reticulum Stress in Parkinson'S Disease: Insights Into Cellular Dysfunctions." *Inflammation*.

Zhang, S., L. Wang, S. Yi, et al. 2025. "Drosophila Aux Orchestrates the Phosphorylation-Dependent Assembly of the Lysosomal V-ATPase in Glia and Contributes to SNCA/α-Synuclein Degradation." *Autophagy* 21: 1–20.

Zhang, S., S. Yi, L. Wang, et al. 2023. "Cyclin-G-Associated Kinase GAK/dAux Regulates Autophagy Initiation via ULK1/Atg1 in Glia." *Proceedings of the National Academy of Sciences of the United States of America* 120: e2301002120.