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Synphilin-1 and its Effects on Pathogenesis of Parkinson's Disease

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Abstract:

Parkinson's Disease (PD) is a progressive neurodegenerative and movement disorder primarily caused by the degradation of dopaminergic neurons. Known markers of neurodegeneration in PD are Lewy Bodies, which are fibrillar aggregates that are found in the brains of PD patients. Lewy Bodies can accumulate from specific mutations in the *SNCA* gene that codes for alpha-synuclein, a protein enriched in presynaptic neurons. A mutated *SNCA* gene can cause conformational aggregates of alpha-synuclein to form toxic species mediating neuronal death. Research into alpha-synuclein has led to the discovery of a binding partner known as synphilin-1 that is also found in protein aggregates including Lewy Bodies in neurons of PD patients. Proteasomal pathways and autophagy are known to control the degradation of alpha-synuclein and synphilin-1 to moderate this cellular toxicity. How the cytosolic inclusions of synphilin-1 and alpha-synuclein are related to changes in the degradation of these proteins is debated. Furthermore, little is known about the physiological function of synphilin-1 or its role in pathogenesis. This literature review will synthesize existing research regarding synphilin-1's role in degradative pathways and establish future avenues for research.

Introduction:

Parkinson's Disease (PD) is a progressive neurodegenerative disorder resulting from the degradation of dopaminergic neurons. Deficiency in dopamine production can lead to chronic motor problems such as tremors and rigidity. Non-motor problems may also persist and include cognitive impairment. Mostly sporadic in nature, PD cases can also come in familial forms from mutations in a variety of extensively studied genes like *SNCA/PRKN1*, *PRKN2*, PTEN-induced putative kinase 1 (*PINK1*), Daisuke-Junko1 (*DJ1*), and leucine-rich repeat kinase 2 (*LRRK2*) (Ganguly et al. 2018). These mutated genes contribute to disruption of autophagy and the proteosome. Autophagy plays the role of clearing misfolded protein aggregates using double membraned, autophagic vacuoles (Glick et al. 2010). The proteosome also clears ubiquitinated misfolded proteins (Lim et al. 2007). While existing treatments control motor symptoms, there are still no therapeutics that help stop the progression of the disease. Research is developing to find new ways to target genetic markers and mechanisms on the molecular level to halt the onset and progression of PD. Some of this research stems from the molecular physiology of a protein known as alpha-synuclein that is encoded by *SNCA*. The expression levels and mutations of *SNCA* play critical roles in the pathogenicity of PD.

Alpha-synuclein is enriched in presynaptic terminals and has been shown to aggregate in the form of Lewy Bodies, which are known to be a histological hallmark of PD that contributes to some level of cell toxicity in PD patients. They can be widely distributed in the central nervous system, especially dopaminergic neurons that have a significant impact on the pathology of PD (Gomez-Benito et al. 2020). Overexpression of *SNCA* has been shown to make dopaminergic neurons vulnerable to degradation (Tagliafierro et al. 2016). Transgenic mouse lines overexpressing *SNCA* have motor impairments and decreased neurotransmission of

dopamine (Tagliafierro et al. 2016). An increase in Lewy Bodies in the brains of PD patients can also be a result of specific point mutations in the *SNCA* gene that affect alpha-synuclein's quaternary structure. A mutated *SNCA* gene results in a misfolded alpha-synuclein protein that is more susceptible to forming toxic oligomers or aggregates (Gomez-Benito et al. 2020). Alpha-synuclein's physiological role is still unclear, but it is highly involved in the progression of PD in patients.

Research into alpha-synuclein has led to the discovery of the binding partner synphilin-1 that is also found in protein aggregates including LBs in neurons of PD patients. Synphilin-1 overexpression can lead to accumulation of synphilin-1 aggregates (Engelender et al. 1999). Since synphilin-1 is an intrinsic component of Lewy Bodies, its association with alpha-synuclein highlights a potentially pathological role (Ribeiro et al. 2002). Like alpha-synuclein, synphilin-1 is widely distributed in the central nervous system and its physiological role is uncertain. It is hypothesized that synphilin-1 may have its synaptic roles modulated by alpha-synuclein during synaptic vesicle trafficking (Burré et al. 2015). In addition, synphilin-1 has both cytoprotective and cytotoxic effects in neurons when binding to alpha-synuclein that are being currently investigated. Experimental data has shown that misfolded synphilin-1 proteins that aggregate pose cytotoxicity in neuronal cells, yet Lewy Bodies in association with wild type synphilin-1 can serve to help eliminate toxic syphilin-1 aggregates (Tanaka et al. 2004). Clearly, there is conflicting data on synphilin-1's physiological role as research in PD pathogenesis continues.

A balance between synthesis and degradation of cellular proteins is necessary for cell survival, and this is done through proteasomal pathways and autophagy. In the case of PD, proteasomal pathways and autophagy are known to clear alpha-synuclein aggregates as well as synphilin-1 to moderate the cell toxicity in PD patients. The ubiquitin proteosome pathway

utilizes ligases to ubiquitinate unwanted proteins as shown in **Figure 1**. In the case of PD, two of the most important E3 ligases that exists in eukaryotic cells are SIAH-1 and SIAH-2 that ubiquitinate synphilin-1 and alpha-synuclein for it to be degraded by the proteosome (Liani et al. 2004). Overexpression of synphlin-1 in transgenic mice can actually promote proteostasis of alpha-synuclein (Casadei et al. 2014). In addition to the ubiquitin-proteosome pathway, autophagy also serves the purpose of removing protein aggregates that accumulate in the cell (Zhang et al. 2012). Autophagy is activated upon cellular stress. In general, the three types of autophagy are macroautophagy, microautophagy, and chaperone mediated autophagy (CMA). Macroautophagy is a multistep process that forms double membrane structures called autophagosomes that fuse with lysosomes containing proteins to be degraded. Microautophagy is the selective degradation of intracellular constituents that are directly invaginated by lysosomes. Chaperone mediated autophagy uses chaperones that recognize target proteins to be delivered to lysosomes to be degraded (Zhang et al. 2012). Both the proteosome and autophagy are important factors in regulating PD as they serve to modulate synphilin-1 levels that may contribute to pathogenesis.

Synphilin-1 can form aggregates while interacting with alpha-synuclein to promote the progression of disease in PD patients. While its mechanisms are not fully understood, synphilin-1 has a significant physiological role. In addition to its effects on intracellular degradation, synphilin-1 can form enough cytosolic inclusions to impair essential dopaminergic neuronal synapses that are responsible for several motor and nonmotor functions (Engelender et al. 1999). Synphilin-1 also demonstrates ambiguous functions when interacting with alpha-synuclein that either enhances or worsens cell survival. Elucidating synphilin-1's impacts will have to start from understanding its discovery, its interactions with alpha-synuclein, and how it is involved in

autophagy and the proteosome. This literature review will highlight these important aspects of synphilin-1 in relation to PD pathology.

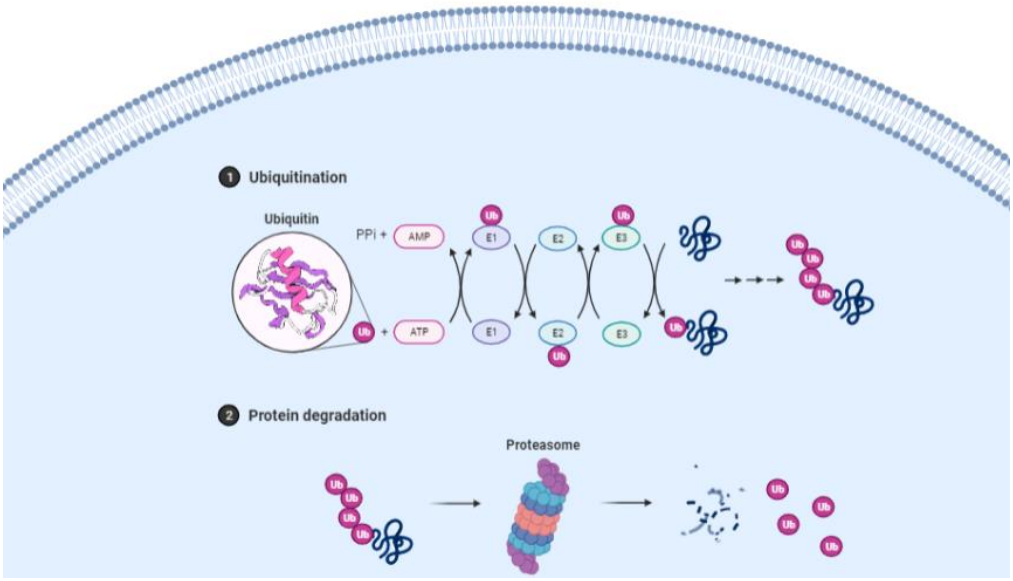

Figure 1. Ubiquitin-Proteosome system is responsible for the degradation of ubiquitinated proteins: the first diagram shows E1 and E2, where E2 is the ubiquitin-conjugating enzyme to which the ubiquitin is transferred from the E1. E3 is the ubiquitin ligase that ubiquitinates the target protein. This ubiquitinated protein gets degraded by the proteasome. Illustration created with BioRender.com.

Section I: Parkinson's Disease and Alpha-synuclein's Contribution to Pathogenesis

Parkinson's Disease (PD) has several pathological markers and clinical features not fully understood. A common neurodegenerative disease second only to Alzheimer's disease, PD has a high prevalence among the older population. Tremors, rigidity, and bradykinesia are some of the most prevalent symptoms that older PD patients can experience. Before PD pathologically progresses into severe clinical symptoms, there is a premotor or prodromal phase of the disease (Kouli et al. 2018). The prodromal phase of PD has been shown to start at peripheral structures of the nervous system causing a host of symptoms including constipation, restlessness, rapid eye

movement, and depression. These symptoms may last for several years and often go undiagnosed as PD until motor symptoms arise. The prodromal state of PD is perceived as an ideal time for therapeutic intervention as it is such an early stage of the disease that it has not impacted the motor sensory neurons that cause the tremors, rigidity, and bradykinesia. However, clinical diagnosis of PD generally occurs at the time the patient presents with motor problems. Currently, the best tool medical practitioners use to diagnose PD is the onset of motor problems and patient response to levodopa treatment (Armstrong et al. 2020). More improvements in alternative treatments and early diagnosis of symptoms are being pursued every day.

The etiology of PD is multifactorial, having both genetic (familial) and environmental (sporadic) factors contributing to the progression of the disease. Familial and sporadic PD have been shown to lead to similar phenotypes. Most cases are sporadic but about 15% come familial and have generally the same progression of neurodegeneration (Armstrong et al. 2020). Specific genetic mutations in PARK genes have been identified to potentially contribute to PD pathogenesis. These PARK genes include *SNCA/PARK1* (encoding alpha-synuclein), *PARK2* (Parkin), *PINK1*, *DJ-1*, and *LRRK2*. The rest of the cases that form sporadic PD may come from factors such as age, smoking, and diet. Thus, the environment is a major contributor to PD. Age is the biggest environmental risk factor, with a median age of 60 having onset of PD. Cohort studies have shown pesticides, herbicides, and heavy metals to significantly contribute to onset of PD, while the mechanisms to how they do so are not fully understood.

Besides the motor symptoms mentioned previously, PD has several pathogenic impacts to the cell including mitochondrial dysfunction, defective protein clearance mechanisms, and neuroinflammation. Many of these processes can be understood from knowing how alpha-synuclein operates in dopaminergic neurons. Alpha-synuclein and Lewy Body formation can be

found both in the central nervous system and peripheral nervous system (Stefanis et al. 2012). The native structure of alpha-synuclein is soluble and mostly unfolded and thus not so susceptible to aggregation (Ostrerova-Golts et al. 2000). However, misfolding can cause alpha-synuclein to adopt a beta sheet rich amyloid structure that is predominantly found in Lewy Bodies. Amyloid structures are secondary structures held by hydrogen bonding and come in fibrillar aggregates to cause neurodegenerative disorders (Gómez-Benito et al. 2020). Misfolding may occur due to the well-studied A30P and A53T point mutants of alpha-synuclein. Aggregation of mutated alpha-synuclein has been found to be more pronounced in A53T mutants than A30P mutants in transfected BE-M17 neuroblastoma cells, but cellular mechanisms work to clear the toxicity resulting from these proteins (Ostrerova-Golts et al. 2000). The two major clearance systems humans use are the ubiquitin-proteosome pathway that ubiquitinates abnormal proteins for degradation by the proteosome and autophagy, which is the process of delivering organelles or damaged proteins engulfed in double-membraned vesicles to lysosomes (Kouli et al. 2018). There are clearly several events impacted by PD which hinder normal function of a dopaminergic neuronal cell.

Alpha-Synuclein may be modified by phosphorylation, oxidation, nitrosylation, glycation, or glycosylation. Of all such modifications, the best studied is phosphorylation. Phosphorylation of alpha-synuclein at S129 was observed to contribute to alpha-synuclein mediated toxicity in cells of PD patients (Li et al. 2020). However, there has been some conflicting evidence to suggest a protective role for phosphorylated alpha-synuclein. Either way, the presence of phosphorylated alpha-synuclein is greater in the brains of PD patients than those of healthy brains. This phosphorylation has also been shown to modulate membrane binding by alpha-synuclein (Stefanis et al. 2019). Posttranslational modifications of alpha-synuclein prove

to be another important area of study in PD pathogenesis besides the A53T and A30P point mutations of alpha-synuclein due to the implications phosphorylated alpha-synuclein has on degradation of dopaminergic neurons.

Section II: The Discovery of Synphilin-1

Alpha-synuclein is clearly an important component of Lewy bodies, but the discovery of synphilin-1 has opened new pathological inquiries for PD. Synphilin-1 was found to be an intrinsic component of Lewy bodies while acting as a binding partner to alpha-synuclein. This interaction may be a key element to the aggregation of cytosolic inclusions and the eventual degradation of dopaminergic neurons (Engelender et al. 1999). The regions of synphilin-1 that were most notably found to bind to alpha-synuclein were the N terminus and its central region. Deleting the first 349 amino acid residues of the N terminal region of synphilin-1 abolishes its interaction with alpha-synuclein (Engelender et al. 1999). This study went on to demonstrate the colocalization of alpha-synuclein and synphilin-1 in neurons.

The *SNCAIP* gene that encodes synphilin-1 has been mapped out through extensive research (Engelender et al. 1999). The *SNCAIP* locus is within the q arm of chromosome number 5 and contains polymorphic repeats of GT nucleotides. The *SNCAIP* gene has an open reading frame containing 10 exons. Exons 4 through 7 encode ankyrin-like repeats and the coil domain of synphilin-1. There is also a GTP and ATP binding domain in synphilin-1. When bound to ATP or GTP, synphilin-1 is more prone to aggregate and bind to alpha-synuclein (Engelender et al. 2000). The data collected on synphilin-1's structure contributes to an understanding of its physiological role that is continually researched.

Subsequent experiments demonstrating syphilin-1's interaction with alpha-synuclein came from co-immunoprecipitation experiments with glutathione S-transferase (GST) fusion proteins (Engelender et al. 1999). Wild-type, A30P, and A53T mutated alpha-synuclein GST fusion proteins were incubated with HEK 293 cells transfected with synphilin-1. Synphilin-1 co-precipitated with alpha-synuclein to confirm their interactions with each other, but synphilin-1 did not interact with a GST protein alone. Co-transfection of constructs encoding synphilin-1 and a portion of alpha-synuclein in HEK 293 cells has also been found to cause the formation of cytosolic inclusions. (Engelender et al. 1999). These experiments suggest synphilin-1 is a direct binding partner to alpha-synuclein.

Further research has looked into synphilin-1's interaction with alpha-synuclein amidst synaptic vesicle trafficking between dopaminergic neurons. Synphilin-1 is already known to be a regulator of synaptic vesicle trafficking (Burré et al. 2015). Some experiments have measured the degree of association between synaptic vesicles and synphilin-1 by immunoprecipitation. Synphilin-1 is strongly associated with synaptic vesicles and in vitro binding experiments have shown this association to be resistant against high salt washing (Ribeiro et al. 2002). Because synphilin-1 is found to have co-immunoprecipitated with synaptic vesicles, there may be an important role for synphilin-1 to direct vesicular structures across synapses. However, what is not well understood is how synphilin-1's influence on synaptic vesicles would be impacted by alpha-synuclein and its mutated forms (Burré et al. 2015). Previous claims have suggested alpha-synuclein anchors synphilin-1 to synaptic vesicles, but levels of immunopurified alpha-synuclein on these vesicles were undetectable (Ribeiro et al. 2002). Purified alpha-synuclein added to a mixture of synaptic vesicles and synphilin-1 however showed that alpha-synuclein, both wild type and A53 mutant, can modulate synphilin-1 binding with vesicles. Alpha-synuclein was

discovered to break synphilin-1's association with synaptic vesicles. Thus, synphilin-1's association with synaptic vesicles is negatively modulated by alpha-synuclein (Ribeiro et al. 2002). Alpha-synuclein may have a way of modulating synaptic vesicle trafficking while interacting with synphilin-1. Some of the effects alpha-synuclein can have in modulating synaptic vesicle trafficking were discovered to include loss of presynaptic proteins, decrease of neurotransmitter release, redistribution of SNARE proteins, enlargement of synaptic vesicles, and inhibition of synaptic vesicle recycling (Burré et al. 2015). Synaptic vesicle release is another significant pathological indicator of PD caused by alpha-synuclein's interaction with synphilin-1.

Section III: Clearance of Aggregated proteins by Autophagy and the Proteosome

Autophagy and the proteosome are central players that modulate synphilin-1 levels in cells of PD patients. Both autophagy and the proteosome accomplish the task of degradation of protein aggregates, but there is a contrast in the type of proteins they target. Autophagy is seen to contribute to the degradation of materials that are larger in size while the proteasome handles degradation of short-lived proteins (Lilienbaum et al. 2013). Autophagy is regarded as a way for cells to combat stress in a variety of situations and they use key regulatory proteins to manage them. mTOR, a protein complex that maintains balance between cellular catabolism and anabolism, is one of these master regulatory proteins that inhibits autophagy through its inhibitory effects on a protein kinase known as Ulk1 (Zhu et al. 2019). Ulk1 initiates macroautophagy and drives formation of autophagosomes (Yan et al. 2017). Autophagy is increased in cells under nutrient deprivation and other stresses. The ubiquitin-proteosome system consists of two steps: ubiquitination and proteasomal degradation. Ubiquitination is carried out through an enzymatic cascade that attaches ubiquitin to target proteins (Liani et al. 2004). The

attached ubiquitin acts as a signal and are targeted to the proteosome that acts as a major pathway for degradation of cytosolic, nuclear and membrane target proteins in all eukaryotic organisms (Livneh et al. 2016). SIAH-1 and SIAH-2 ligases are among the most relevant E3 ligases that ubiquitinate alpha-synuclein and synphilin-1. SIAH-1 is primarily responsible for the ubiquitination of synphilin-1 whereas SIAH-2 is responsible for ubiquitination of alpha-synuclein (Liani et al. 2004). Clearing aggregates through the ubiquitin-proteosome system and autophagy holds tremendous capabilities in halting PD pathogenesis.

Autophagy is critical in PD patients, as there is found to be an accumulation of autophagic vacuoles in the brain when there is an accumulation alpha-synuclein (Winslow et al. 2010). Insufficient fusion of these vacuoles with lysosomal compartments is a leading explanation to the abundance of cellular toxins in the cytosol of neuronal cells. Mice with genetically downregulated autophagy proteins have been shown to exhibit the same neurodegenerative patterns as PD patients, thus suggesting the neuroprotective role autophagy has against pathogenesis (Wan et al. 2020). Several experimental models explain the protection autophagy offers for cell survival. Some of these models include using animals to induce cytotoxicity in cells to activate autophagy with specific compounds (Zhang et al. 2012).

Some experiments have shown the capabilities of autophagy to clear alpha-synuclein from cells. For example, a stable PC12 cell model with induced macroatuophagy was found to degrade A53T mutated alpha-synuclein (Webb et al. 2003). Wild type alpha-synuclein and an A30P mutant of alpha-synuclein were also degraded but mostly through the proteosome (Webb et al. 2003). Pharmocological induction of macroautophagy with drugs like rapamycin is also capable of degrading wild type, A53T, and A30P mutated alpha-synuclein (Stefanis et al. 2019).

Autophagy is an important line of defense against alpha-synuclein aggregates, hence why research in this field could pave way for novel autophagy induced treatments for PD patients.

CMA is another primary pathway alpha-synuclein is cleared within cells, and any induction of this pathway has therapeutic advantages to PD patients. Alpha-synuclein is recognized by chaperones when they are destined for degradation by CMA (Tofaris et al. 2003). With the assistance of chaperones, alpha-synuclein binds lysosomal-associated membrane protein type 2A (LAMP-2A). Lysosomes are able to uptake alpha-synuclein for degradation, and it has been demonstrated that knockouts of LAMP-2A leads to an increase of cytosolic alpha-synuclein (Martinez-Vicente et al. 2008). While CMA has ways of degrading alpha-synuclein, there are some factors like mTOR that can downregulate chaperone mediated autophagy. Pharmacologically inhibiting mTOR would help increase CMA and lower alpha-synuclein aggregates in the cell (Moors et al. 2017). In brief, we can see CMA offers another opportunity for alpha-synuclein to be degraded.

Dysfunction in the proteosome has been implicated in PD as well. Alpha-synuclein evidently promotes the dysfunction of the proteosome which can lead to the further accumulation of alpha-synuclein aggregates (Lim et al. 2007). A dysfunctional ubiquitin-proteasome system has been shown to lead to dopaminergic neuronal death (Lim et al. 2007). Clearing toxic species that result from accumulated alpha-synuclein aggregates can increase ubiquitin-proteasomal activity and ensure proper disposal of ubiquitinated proteins.

Section IV: Inhibition of Autophagy and the Proteosome due to Alpha-Synuclein

Accumulation of wild type and mutated alpha-synuclein has been shown to disrupt autophagy and has been determined to be done through the influence over Rab1a. Rab1a is a

small GTPase protein that regulates vesicle trafficking, including in autophagy. Alpha-synuclein has been determined to disrupt autophagy, but additional studies have shown its ability to inhibit Rab1a to impact a cell's secretory pathway (Winslow et al. 2010). Alpha-synuclein overexpression experiments have been shown to decrease autophagy based on lower levels of LC3-II. LC3-II, a soluble microtubule-associated protein, comes from an LC3-I conjugated to phosphatidylethanolamine that is recruited to autophagosomes. LC3-II is a reliable marker for autophagy because it is involved in the final stages of autophagosome maturation, and is recycled during lysosomal degradation (Tanida et al. 2008). While research into alpha-synuclein overexpression has been determined to decrease vesicle count, additional studies have pursued the effect of co-overexpression with Rab1a (Gitler et al. 2008). If Rab1a is overexpressed in a cell that has impaired autophagy from overexpression of alpha-synuclein, autophagy is rescued and aggregates of alpha-synuclein in the cytoplasm were found to be degraded (Winslow et al. 2010). Rab1a therefore has significant implications in PD patients with overexpressed alpha-synuclein.

Alpha-synuclein was also found to increase levels of insoluble p62 proteins. p62 is a classical receptor in autophagy and is involved in the proteasomal degradation of proteins. (Shin et al. 2020). p62 can colocalize with LC3 during autophagy. LC3-II conversion triggers autophagosomes to engulf target proteins. p62 turnover and protein amount is a reliable source in measuring the activity of autophagy. (Tanida et al. 2008). However, p62 is not able to colocalize with autophagosomes with overexpressed alpha-synuclein and results in a decrease in vesicle count (Shin et al. 2020). Alpha-synuclein therefore has an impact on autophagy through its effects on p62 proteins.

Additional experiments have studied Atg9 as another protein to induce autophagy. Atg9 is an autophagy related protein that directs membranes from donor organelles to form autophagic vacuoles (Winslow et al. 2010). Atg9 normally localizes with the trans-Golgi network, but autophagy induced cells trigger Atg9 localization with LC3-II positive autophagosomes. Studies that overexpressed alpha-synuclein found that Atg9 was mislocalized in the cell, and autophagosome vesicles were greatly reduced (Lynch-Day et al. 2010). A model supports that Rab1a functions through Atg9. The consensus is that alpha-synuclein will inhibit Rab1a activity and Atg9 colocalization. Rab1a is thought to operate upstream in autophagy formation, and consequently have influence over Atg9. Experiments show that overexpression of alpha-synuclein and a knockdown of Rab1a will mislocalize Atg9 and cause fewer LC3-II vesicles (Winslow et al. 2010). Atg9 is an important protein that needs to be localized properly for mediating cell toxicity from alpha-synuclein.

Although alpha-synuclein overexpression has been shown to decrease autophagy, studies have gone to observe the effect on autophagy from A53T and A30P point mutants of alpha-synuclein. The A30P mutated alpha-synuclein specifically is known to inhibit chaperone mediated autophagy (Lei et al 2019). One of the foundational pieces of knowledge on autophagy pathways is when one is impaired, another compensatory autophagy pathway is upregulated. Thus, experiments have shown that macroautophagy is upregulated upon impairment of chaperone mediated autophagy (Lynch-Day et al. 2010). The exact mechanism of chaperone mediated autophagy inhibition done by alpha-synuclein has to do with a transcriptional repressor ZKSCAN3. ZKSCAN3 represses transcription of several autophagy proteins including LC3, Ulk1, and WIPI2 and its activation contributes to significant loss of autophagosomes and

autophagy markers like p62 (Lei et al. 2019). Thus, alpha-synuclein may be an enhancer of ZKSCAN3 expression.

Alpha-synuclein can not only exert inhibitory effects on autophagy but the proteosome as well. Proteasomal impairment inhibition by wild type and mutated alpha-synuclein has been speculated as either an allosteric or noncompetitive inhibition of the chymotrypsin like 20S protein core of the proteosome. **Figure 2** depicts the result of misfolded proteins on the proteosome ubiquitin system. The proteosome in dopaminergic cells is largely made up of 26S proteosomes with 20S proteasomal subunits that have a binding affinity towards alpha-synuclein (Zondler 2017). While the exact method of binding to 20S proteasomal proteins is unclear, the amyloid structure of alpha-synuclein has a significant influence in its capabilities of inhibiting proteostasis.

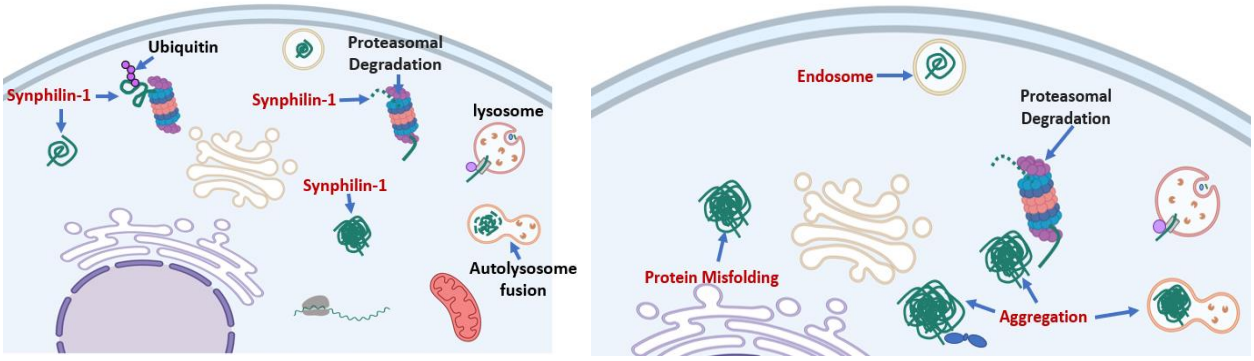

Figure 2 Proteostasis is impaired when protein aggregates accumulate: The diagram at the left hand side depicts proper degradation of protein aggregates with synphilin-1. Synphilin-1 proteins are depicted in green and are ubiquitinated by the purple circles drawn in the diagram. They are then degraded by the proteasome. Autophagosomes and lysosomes are also depicted in the diagram at the left hand side with synphilin-1 lysed in the containing vesicle. The diagram at the right hand side depicts protein misfolding and aggregation in green. These misfolded proteins are shown to accumulate in the cytosol when proteostasis is impaired. Some of these aggregates can accumulate in autophagosomes as well. This illustration was created by Nadine Lebek.

Section V: Drug Treatments

Novel drug treatments are currently being pursued to halt the progression of PD in patients. The drug treatments that impact autophagy affect certain intermediates of the autophagy pathway. As discussed earlier, alpha-synuclein inhibits the proteosome, CMA, and macroautophagy, leading to further accumulation of alpha-synuclein aggregates. Different drug treatments can intervene at certain biochemical pathways to disrupt or promote autophagy. Most of these drug treatments discovered to modulate autophagy manipulate mTOR. mTOR is the master regulator of autophagy by its inhibitory effects on Ulk1 kinase. However, AMPK, a key enzyme that maintains cell homeostasis, can activate Ulk1 and inhibit mTOR to promote macroautophagy. AMPK is known to increase autophagy levels in cells in the presence of higher ADP levels (Zhu et al. 2019). Rapamycin is one drug that acts on autophagy by targeting mTOR. Rapamycin's inhibitory effect on mTOR gives opportunity for autophagy to proceed by activated kinase Ulk1. Aggregates of alpha-synuclein can therefore be degraded in rapamycin treated cells and lower the risk of dopaminergic neuronal loss in PD patients. Rapamycin has been found to decrease the expression of alpha-synuclein, likely from the effects of upregulated autophagy (Zhu et al. 2019). Little data exists on rapamycin treatment effects on synphilin-1. Other drug treatments that have been researched to have influence on certain steps of the autophagy pathway are chloroquine and Metformin.

Metformin serves as a viable therapeutic strategy for PD by inducing autophagy. Experiments have shown Metformin to decrease phosphorylation of alpha-synuclein in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treated mice that would otherwise contribute to cell toxicity and dopaminergic neuronal loss (Sportelli et al. 2020). Metformin can induce autophagy from increasing AMPK activity levels while inhibiting mTOR. Consequently, LC3-II has been

shown to increase and p62 protein levels decrease in response to Metformin treatments in cultured MN9D cells (Yan et al. 2017). Metformin has also been shown to fix mitochondrial impairment. One of the symptoms of PD is mitochondrial dysfunction, which leads to the production of toxic reactive oxygen species that destroys the host cell (Mor et al. 2020). A small degree of mitochondrial impairment has been shown to be linked to deficient E3 ligase activity from SIAH-1 which is responsible for ubiquitinating synphilin-1 (Dawson et al. 2010). Metformin has been suggested to reduce reactive oxygen species, but has also been studied to induce autophagy indirectly by acting on Complex I of the electron transport chain in the mitochondria. Metformin inhibits Complex I which results in reduced ATP production by ATP synthase, thus activating AMPK (Mor et al. 2020). Metformin inhibits phosphorylation of alpha-synuclein by several kinases such as G-coupled protein receptors and casein kinases. Since phosphorylated alpha-synuclein has been shown to lead to cell toxicity, Metformin would decrease the further accumulation of alpha-synuclein aggregates. Little data exists on Metformin's effect on synphilin-1, but there is data that suggests that phosphorylated synphilin-1 can promote autophagy (Li et al. 2020). More research needs to be conducted to explore phosphorylated synphilin-1's effects on autophagy, but Metformin is considered a viable drug treatment to increase autophagy in PD patients.

Chloroquine is known to reduce autophagy by acting on lysosomal pH. It will raise the pH of lysosomes that are predestined to form autophagosomes (Mauthe et al. 2018). Lysosomes that have a pH raised too high are unable to properly degrade proteins, causing complications in the cell such as contributing to a toxic environment. Chloroquine is often used to mimic dysfunctional autophagy in a cell and the results are comparable to that of a cell with

accumulated alpha-synuclein aggregates (Gao et al. 2019). Chloroquine serves as a viable positive control for most experiments to analyze the impact of impaired autophagy.

Future Directions

Further experiments need to understand alpha-synuclein and its interactions with synphilin-1 *in vivo* and *in vitro* with drug treatments. Synphilin-1's overarching effects on autophagy under chloroquine, Metformin, and rapamycin drug treatments are still unknown as well. There needs to be a comprehensive model demonstrating which drugs have an effect on synphilin-1 or vice versa. There should also be future experiments measuring cell death according to different expression levels of synphilin-1 under each drug treatment. mTOR is important in maintaining cellular homeostasis while regulating autophagy but little is known about synphilin-1's influence over mTOR. More research needs to be conducted to see whether overexpressed synphilin-1 acts on mTOR as it does on AMPK. If overexpression of synphilin-1 activates AMPK, does it inhibit mTOR to yield upregulated autophagy? Further studies into their physiological roles within dopaminergic neurons and identification of their binding partners is important treating PD patients successfully. There are also lingering questions on the neuroprotective capabilities of synphilin-1 against mutated forms of alpha-synuclein. Alpha-synuclein aggregation is an unclear area of study in PD, as the mechanisms of how the aggregates form and the structure of A53T and A30P mutants of alpha-synuclein is still being researched. By furthering studies into synphilin-1 and alpha-synuclein's effects on the several mechanisms involved in pathogenicity of PD patients, improved and novel therapeutics can be administered.

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