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An Examination of Post-Translational Modifications of α-Synuclein and its Effects on Parkinson's Disease

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ABSTRACT

Parkinson's disease (PD) is the second most common degenerative disease of the central nervous system. PD affects millions of people worldwide, so it is critical to research and understand Parkinson's disease to better help those affected. The exact cause and pathogenesis of PD is unknown, but the aggregation of α-Synuclein into Lewy bodies has been associated with PD. a-Synuclein often goes through post-translational modifications (PTMs). PTMs can affect the shape, localization, function, and activity of proteins like a-Synuclein. PTMs of a-Synuclein can also affect how the protein aggregates. PTMs of α-Synuclein can play a big role in the pathogenesis of PD, and understanding how PTMs affect α-Synuclein is critical in helping forward research that focuses on PD. This paper discusses the effects of various PTMs on α-Synuclein and how they affect the pathogenesis of Parkinson's Disease.

Introduction

Parkinson's Disease (PD) is estimated to affect as many as 1 million Americans and 10 million people worldwide (Parkinson's Foundation). Additionally, more than 90,000 people in the U.S. are diagnosed each year with this movement disorder. PD is characterized by a degeneration of nerve cells in the substantia nigra, a critical dopamine producing region of the brain. The substantia nigra also controls movement. This degeneration of nerve cells in PD leads to motor symptoms, as well as non-motor symptoms. While the exact cause of PD is unknown, researchers believe that PD is caused via a combination of genetic and environmental factors. Among those factors is α-Synuclein, a neuronal protein that is linked genetically and pathologically to PD. Furthermore, PD is associated with the aggregation of these proteins into intracellular inclusions called Lewy Bodies. This aggregation of α-Synuclein could be a factor of neuron degeneration in PD. (Stefanis, 2011) Genetic mutations of α-Synuclein are often associated with PD pathogenesis, but new research has shown that post-translational modifications (PTMs) of α-Synuclein also play an important role in the pathogenesis of PD. Post-translational modifications of proteins refer to covalent, chemical modifications of polypeptide chains that can happen after mRNAs are translated into proteins. PTMs can modify the properties of proteins through the addition of functional groups, such as phosphoryl, acetyl, hydroxyl, and methyl, to amino acids. PTMs can also occur through the hydrolysis of peptide bonds between amino acids, leading to the breakdown of proteins into smaller amino acids or polypeptides. This process is known as proteolytic cleavage or proteolysis. PTMs are important because chemical modifications and proteolysis can change the physical and chemical properties of a protein such as its activity, localization, stability, shape, and function. (Ramazi et al. 2021) Post-translational modifications of α-Synuclein could play a major role in the pathogenesis of Parkinson's Disease. Understanding how post-translational modifications of α-Synuclein affect the protein and its role in the pathogenesis of Parkinson's Disease

is critical in developing the current understanding of PD, as well as furthering research regarding possible therapies. This examination will discuss post-translational modifications of α-Synuclein, their effect on the properties of α-Synuclein, and how they affect the pathogenesis of Parkinson's Disease.

The Structure of α-Synuclein

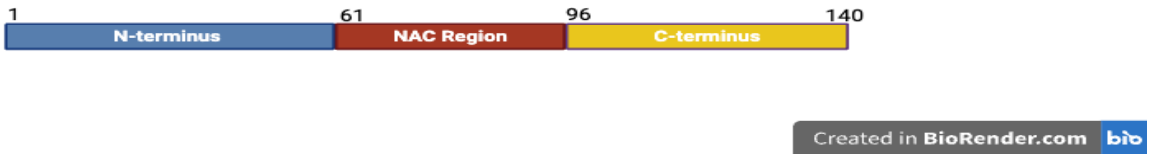

Figure 1. The Structure of α-Synuclein. This figure shows the 3 domains of α-Synuclein: the N-terminus, the NAC region, and the C-terminus. Created in BioRender by Surya Kapu (2024, February 29).

α-Synuclein is an abundant neuronal protein encoded by the SNCA gene, and is composed of 140 amino acids. The protein typically localizes in presynaptic terminals of neurons. The exact structure of α-Synuclein is unclear, but it has been described as an "intrinsically disordered protein" in aqueous solutions (Stefanis, 2011). Understanding the structure of α-Synuclein can provide insight into how the protein goes through post-translational modifications. α-Synuclein is composed of three distinct domains: the N-terminus, the non-amyloid-β component (NAC), and the C-terminus. (Meade et al. 2019)

The amphipathic N-terminus (residues 1-60) contains the KTKEGV sequence and shows 11-mer repetitions. The KTKEGV sequence plays an important role in membrane binding. Upon membrane binding, an amphipathic α-helical structure is formed in the N-terminus. The N-Terminus may be linked with lipid and protein binding. The N-terminus may play a role in the fibril structure of α-Synuclein, as well. Truncation in the N-terminus could impact the formation and morphology of the fibrils in α-Synuclein. A number of other post-translational modifications occur in the N-Terminus such as acetylation and ubiquitination (Bartels et al. 2010).

The NAC domain (residues 61-95) is in the center of the α-Synuclein sequence. The region is hydrophobic with charged residues flanking it. The NAC domain is necessary for the aggregation of α-Synuclein. The NAC domain is responsible for fibrillization of α-Synuclein. The domain can be amyloidogenic, as well. The NAC region makes up the core of some amyloid fibril structures of α-Synuclein. Studies have also indicated that the NAC region is needed for the formation of β-sheets. Glycation and truncation are common PTMs that occur in the NAC domain. (Xu et al. 2016)

The C-terminus (residues 96-140) contains many negatively charged residues. There are a few proposed functions of C-terminus. One proposed function of the C-terminus is a chaperone activity, the hypothesis that the C-terminus in α-Synuclein may inhibit the aggregation of proteins. The C-terminus could also be involved in metal binding. Due to the many negatively charged residues in the C-terminus, the region may be able to bind to a number of metals. This function of metal binding neutralizes its extremely negative charge and thus leads to an increase in aggregation. Additionally, the C-terminus may prevent the aggregation of α-Synuclein through electrostatic repulsion. Due to the highly negative C-terminus region, intermolecular electrostatic repulsion occurs between α-Synuclein molecules. This electrostatic repulsion can be counteracted through neutralization or truncation of the C-terminus region in α-Synuclein. Therefore, neutralized or truncated α-Synu-

clein molecules are much more likely to aggregate. The C-terminus has also been hypothesized to bind to calcium ions or other proteins. Many PTMs also occur in the C-terminus region like truncation and nitration. (Sorrentino et al. 2018)

Post-Translational Modifications

Post-translational modifications (PTMs) are the modifications that proteins undergo after translation and synthesis. To put it another way, chemical changes and modifications are made to a polypeptide chain after RNA has been translated and a protein has been synthesized. PTMs occur when a modifying chemical group is added to the protein. Some examples of these chemical groups are acetyl, methyl, and phosphoryl. Additionally, PTMs, such as prenylation, include the addition of complex molecules. PTMs can also happen via proteolytic cleavage or the addition of polypeptides. In proteolytic cleavage, peptide bonds are hydrolyzed by the enzyme protease. Proteolytic cleavage is irreversible and can result in major changes in a protein's structure and function. PTMs are incredibly important for regulation in organisms and cells. PTMs also lead to a wide diversity of proteins and the proteomes of organisms. PTMs change the physical and chemical properties of proteins like α-Synuclein. PTMs lead to variations in the function, stability, activity, localization, shape, structure, and many other properties in proteins. In the case of α-Synuclein, PTMs also change the way the protein aggregates. Since the properties and aggregation of α-Synuclein are very important characteristics in the pathology of Parkinson's disease, understanding what PTMs affect α-Synuclein, how they affect α-Synuclein, and what role they play in Parkinson's disease is crucial. (Ramazi et al. 2021)

Post-translational modifications can be reversible or irreversible. Reversible PTMs are mainly covalent modifications, while irreversible PTMs are primarily proteolytic modifications or involve chemical processes. PTMs involving the covalent addition of chemical groups, such as phosphorylation or acetylation, are reversible. This addition of chemical groups is controlled by enzymes. Certain deconjugating enzymes can reverse these chemical group additions, too. Modifications in amino acids, such as deamidation, are irreversible because they involve chemical processes rather than covalent modifications and regulation by enzymes. PTMs brought about by cleavage, like proteolysis, are irreversible as well. PTMs via the addition of complex molecules are reversible, and PTMs, such as ubiquitination, brought about by the addition of polypeptides are reversible. This review will go over several reversible and irreversible PTMs of α-Synuclein. (Ramazi et al. 2021)

Phosphorylation

Phosphorylation is one of the most researched and important post-translational modification. Phosphorylation involves the addition of a phosphate group to serine, threonine, tyrosine, proline, histidine, arginine, cysteine, and aspartic acid residues, but phosphorylation primarily occurs on serine, threonine, histidine, and tyrosine residues. During phosphorylation, a phosphate group is transferred via protein kinases from adenosine triphosphate to the receiving residue. Phosphorylation is reversible using phosphatase enzymes that can reverse the reaction in a process known as dephosphorylation. Phosphorylation affects important cell processes such as the cell cycle, apoptosis, replication, translation, and signal transduction pathways. Irregular phosphorylation can lead to a variety of diseases like Parkinson's or Alzheimer's. (Ramazi et al. 2021)

Phosphorylation of α-Synuclein may affect the properties of α-Synuclein, such as its structure, fibrillization, aggregation, and membrane binding. It can also affect the way α-Synuclein bonds to metals and its metal ion affinity. This can lead to alterations in the folding and structure of α-Synuclein. Phosphorylation has also been found to change the conformation of the protein and inhibit its fibrillization. Understanding how phosphorylation affects α-Synuclein can elucidate the pathology of PD. (Kawahata et al. 2022)

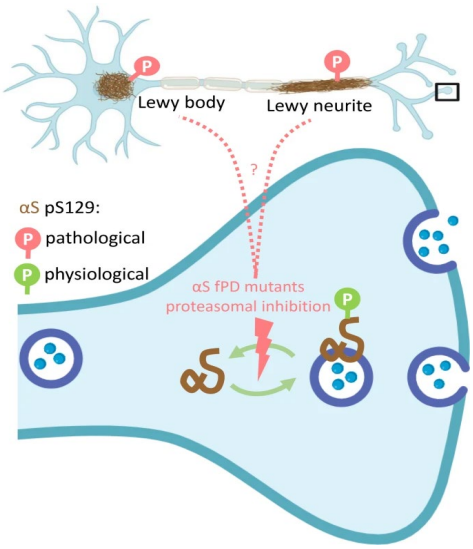

Figure 2. Phosphorylation at S129. Created by Ramalingam and Dettmer (2023).

Phosphorylation of α-Synuclein is linked to the pathogenesis of PD. Experiments have shown that in patients with PD, around 90% of α-Synuclein is phosphorylated at the Ser129 residue. Only 4% of α-Synuclein is phosphorylated in individuals not affected by PD at Ser129. (Xu et al. 2015) Phosphorylation can also happen at some other residues, with a majority of them being in the C-terminal, but the phosphorylation of Ser129 is the most important PTM linked with the aggregation of α-Synuclein and the generation of Lewy Bodies.

Phosphorylation of S129 in α-Synuclein is mediated by kinases such as G-protein-coupled receptor kinases (GRKs). GRKs are serine/tyrosine kinases that regulate G-protein-coupled receptors. Phosphorylation of α-Synuclein via GRKs shows a reduction of α-Synuclein's affinity to bind with phospholipids and membranes, meaning Ser129 phosphorylation inhibits the binding of α-Synuclein to cell membranes. Phosphorylated α-Synuclein prefer localizing to mitochondria. This inhibits cellular respiration and can bring about cell death. Phosphorylation of S129 via GRKs is associated with mitochondrial dysfunction, and plays a significant role in the pathogenesis of PD. Other kinases also mediate S129 phosphorylation such as casein kinase II and polo-like kinase. (Kawahata et al. 2022)

In previous experiments, a paired surface plasma wave biosensor was able to detect phosphorylated Ser129 α-Synuclein in diluted human serum. This indicates that phosphorylated α-Synuclein can be used as a potential biomarker for the diagnosis and observation of PD. (Chen et al. 2022) More research is needed to reach a definitive conclusion, but it looks like phosphorylated α-Synuclein in Ser219 could be a potential biomarker for PD and could be the target of novel therapies.

Acetylation

Acetylation is the addition of an acetyl group from acetyl coenzyme A on a protein's amino acid. Acetylation primarily occurs on lysine residues, but also happens on alanine, arginine, aspartic acid, cysteine, glycine, glutamic acid, methionine, proline, serine, threonine and valine residues. Acetylation can change properties of proteins, such as a protein's hydrophobicity, surface properties, and solubility. This can alter a protein's function, conformation, localization, stability, and aggregation. (Christensen et al. 2019) The most significant type of acetylation is called N-terminal acetylation. N-terminal acetylation is the irreversible addition of an acetyl group via N-terminal acetyltransferase to the amino acids located in the N-terminus region of a protein (Xia et al. 2020). Other types of acetylation are Nε-acetylation and O-acetylation, which are reversible. Nε-acetylation

and O-acetylation are considered reversible as they go through deacetylation, which is facilitated by deacetylase enzymes. N-terminal acetylation, unlike most PTMs dealing with the addition of chemical groups, is considered irreversible because deacetylase enzymes for N-terminal deacetylation have not been found yet. Acetylation contributes to many cell processes like the cell cycle, DNA replication, splicing, transcription, and protein-protein interaction. Abnormal acetylation can lead to diseases such as cancer and Parkinson's disease. (Choudhary et al. 2009)

The acetylation of α-Synuclein plays a critical role in the pathogenesis of Parkinson's Disease. Nearly 80% of α-Synuclein proteins experience acetylation in the N-terminus (Bell et al. 2021). This N-terminal acetylation leads to changes in many of α-Synuclein's properties. First, N-terminal acetylation causes the loss of a positive charge in the N-terminus, and this alters α-Synuclein's structure and its ability to bind to cell membranes. N-terminal acetylation is also believed to be a major factor in the aggregation of α-Synuclein into amyloid fibrils. Furthermore, N-terminal acetylation results in an increased tendency to form a helical structure in the N-terminus. Studies indicate that this increased tendency in forming a helical structure boosts α-Synuclein's resistance to aggregation (Bartels et al. 2014), and that the absence of N-terminal acetylation promotes aggregation of α-Synuclein. More research is needed to determine how N-terminal acetylation affects the localization of α-Synuclein, but studies in yeast have shown that N-terminally acetylated α-Synuclein may localize in the cytoplasm. Additionally, acetylated α-Synuclein showed increased stability and was less inclined to degradation. (Mueller et al 2021) Acetylation is not as widely researched as some other PTMs, but new studies and findings are emerging. Continuing research on the acetylation of α-Synuclein is important and the aggregation resistant qualities of N-terminally acetylated α-Synuclein may pave the way for future therapies.

Truncation

Truncation, an example of proteolytic cleavage, is an irreversible post-translational modification in which a protein's sequence gets shortened by removing a part of it. Truncation contributes to genetic diversity and variation in the human proteome and has the potential to create new proteoforms with different functions. (Fortelny et al. 2015) Protein truncations occur via the activity of an enzyme known as protease. Protease enzymes break down proteins into amino acids or smaller polypeptide chains, and regulate aspects of proteins such as their activity and localization. (Lopez-Otin et al. 2008) Additionally, protein degradation can also lead to a truncated protein. Protein truncation often results in the loss of function in a protein, but some proteins can gain functions. Studying truncated proteins may increase the understanding of the human proteome and genome. Further research on truncation may lead to new therapies for various diseases. Truncated proteins are important for interpreting genomes, and can indicate genes that could be targeted for therapies. (DeBoever et al. 2018)

Truncation of α-Synuclein primarily occurs in the C terminus region, and has a major link to the aggregation of α-Synuclein. About 15% of α-Synuclein aggregated in Lewy Bodies is truncated. The C terminus has structural qualities that limit α-Synuclein misfolding and aggregation. The C terminus is very highly charged and this promotes a disordered structure of α-Synuclein. If the charge in the C terminus were to be neutralized, aggregation of α-Synuclein would occur. Truncation of the C terminus region inhibits these structural qualities, promoting pathological misfolding and aggregation of α-Synuclein. Truncation of the C terminus may also inhibit its chaperone activity, again leading to the aggregation of α-Synuclein. Truncation of α-Synuclein may increase the rate of fibrillization, and contribute to mitochondrial dysfunction in PD. Additionally, research has shown that truncated C termini in α-Synuclein may cause stronger interaction between α-Synuclein and other membranes. Truncation also happens in the N terminus region, however this has a minimal effect on α-Synuclein aggregation. (Zhang et al. 2022)

The primary enzyme associated with the truncation of α-Synuclein is calpain I. Calpain I is a protease that relies on activation via calcium. Proteolytic cleavage facilitated through calpain has been tied to the pathogenesis of many diseases like Alzheimer's. α-Synuclein is thought to be a substrate of calpain. In Parkinson's Disease, calpain is able to affect its pathogenesis by carrying out the truncation of α-Synuclein's C-terminus. This enzyme seems to play a role in the aggregation and pathology of α-Synuclein through proteolysis. The enzymes caspase 1 and neurosin have also been linked to α-Synuclein truncation (Dufty et al. 2007). Targeting these enzymes and inhibiting the truncation of α-Synuclein could be the focus of potential therapies looking to combat α-Synuclein aggregation.

O-GlcNAcylation

O-linked β-N-acetylglucosamine (O-GlcNAcylation) is a reversible post-translational modification characterized by the bonding of N-acetyl glucosamine (GlcNAc) to the serine and threonine residues of a protein. O-GlcNAcylation primarily targets proteins in the nucleus, mitochondria, and cytoplasm. O-GlcNAcylation is regulated by the enzymes O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA). The function of OGT is to add GlcNAc to a protein, and OGA removes GlcNAc from a previously modified protein. The enzyme regulated removal of GlcNAc makes this PTM reversible. The frequency of O-GlcNAcylation is primarily associated with nutrient availability, which also regulates OGT and OGA activity. O-GlcNAcylation levels are regulated into an "optimal zone", a state of homeostasis. Disruption of O-GlcNAcylation homeostasis could contribute to the pathology of many diseases. O-GlcNAcylation affects many protein functions including protein stability, signal transduction, protein-protein interactions, and localization within cells. Abnormal O-GlcNAcylation has been associated with numerous diseases, as well as neurodegenerative disorders. Therapies involving O-GlcNAcylation are currently being researched to combat these diseases. For example, OGA inhibitors are currently being developed to prevent aberrant O-GlcNAcylation. Further research into O-GlcNAcylation could lead to the development of new therapies and illuminate the pathogenesis of many diseases. (Mannino et al. 2022)

New research is showing increasingly emerging links between O-GlcNAcylation and α-Synuclein aggregation. An increasing amount of studies show that O-GlcNAcylation may inhibit α-Synuclein aggregation. Multiple studies have reported that O-GlcNAcylation has protective qualities against α-Synuclein at threonine residues 72,75, and 81 in the NAC region. Moreover, O-GlcNAcylation has been known to inhibit aggregation in aggressive α-Synuclein mutants. O-GlcNAcylation at these residues also dramatically changes the structure of the α-Synuclein aggregation. O-GlcNAcylation also inhibits oligomerization of α-Synuclein, implicating that the PTM could have an effect on α-Synuclein neurotoxicity. O-GlcNAcylation may also affect other PTMs of α-Synuclein, primarily phosphorylation and truncation. For truncation, O-GlcNAcylation inhibits the activity of calpain. This inhibits the cleavage performed by calpain on α-Synuclein. α-Synuclein cleavage leads to aggregation, so inhibiting calpain activity results in less aggregation. Additionally, O-GlcNAcylation has shown to alleviate neurodegeneration and protect dopamine neurons in Parkinson's Disease. O-GlcNAcylation's inhibitory effects on α-Synuclein aggregation, oligomerization, and neurodegeneration indicate its protective nature in the progression of the pathogenesis of Parkinson's Disease. Due to this protective nature of O-GlcNAcylation, further research into therapies targeting O-GlcNAcylation could prove to be beneficial. (Levine et al. 2019)

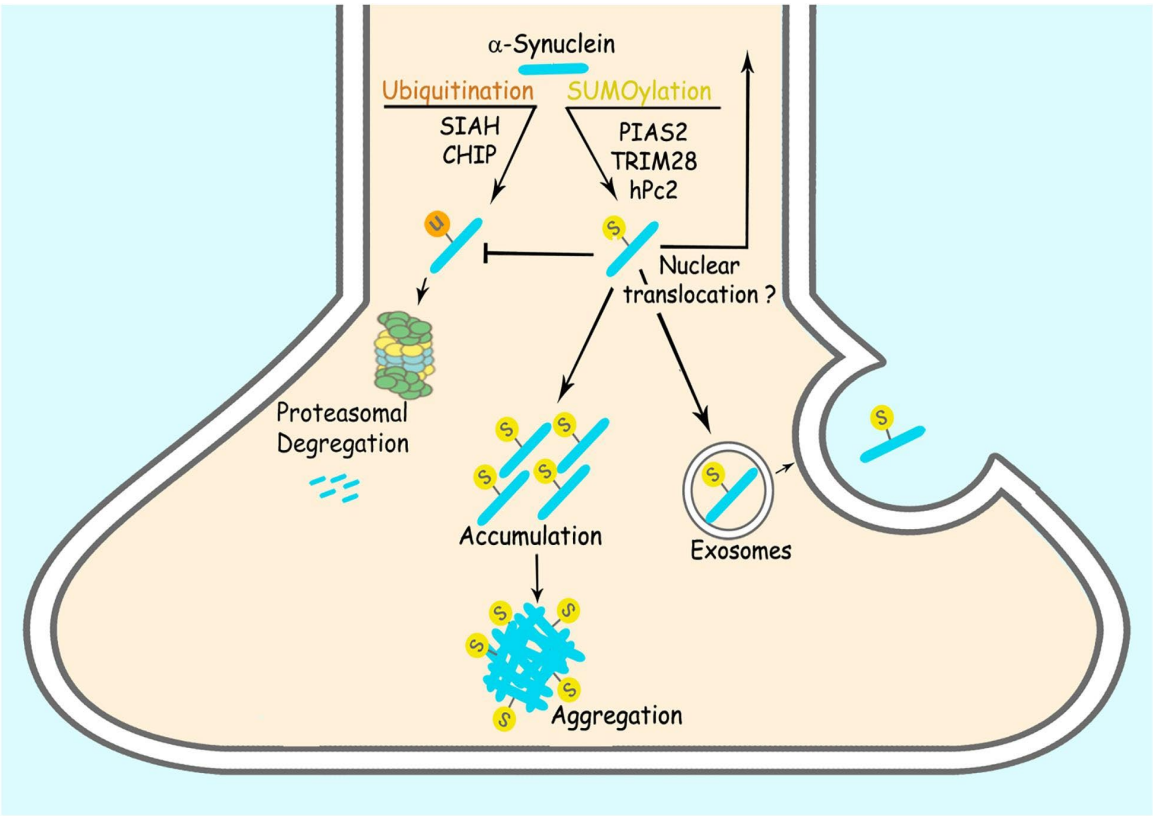

Figure 3. Ubiquitination and SUMOylation of α-Synuclein. Created by Savyon and Engelander (2020).

Ubiquitination

Ubiquitination, sometimes referred to as ubiquitylation, is a reversible post-translational modification involving the addition of the protein ubiquitin to a substrate. Ubiquitin is an abundant protein that regulates cell processes. Ubiquitination is involved in many cell processes, such as apoptosis and protein degradation. Via ubiquitination, ubiquitin can be added to the N-terminus of a target protein to spur proteasomal degradation. Ubiquitination is vital for protein homeostasis. Protein degradation is crucial in organisms to discard unneeded, damaged, or misfolded proteins. Ubiquitination also plays a role in the cell cycle and cell signaling. Ubiquitination is catalyzed by three enzymes: ubiquitin-activating enzymes (E1), ubiquitin-conjugating enzymes (E2), and ubiquitin ligases (E3). First, the E1 enzymes activate ubiquitin through ATP hydrolysis and the adenylation of a glycine residue in ubiquitin's C-terminus. The E2 and E3 enzymes catalyze the bonding of ubiquitin to a lysine residue in a substrate. This process is reversible via deubiquitinating enzymes. Due to ubiquitination's regulatory functions of many cell processes, further research into this reaction could result in potential therapies. For example, scientists are aiming to target and inhibit the E1, E2, and E3 enzymes. Inhibiting these enzymes greatly decreases ubiquitination levels, having major effects in various cell functions. Potential therapies targeting ubiquitination could be used to treat neurodegenerative disorders and cancers. (Guo et al. 2023)

Through immunostaining, multiple studies have found that Lewy Bodies contain α-Synuclein and ubiquitin. This suggests that ubiquitinated α-Synuclein proteins are present in Lewy Bodies. These α-Synuclein proteins in Lewy Bodies are primarily monoubiquitinated, but occasionally polyubiquitinated. Monoubiquitinated α-Synuclein go through proteasomal degradation, and polyubiquitinated α-Synuclein undergo lysosomal degradation. Monoubiquitination of α-Synuclein is catalyzed by the enzyme seven in absentia homolog (SIAH)

at the protein's lysine residues. SIAH is a type of ubiquitin ligase (E3), and has been seen in Lewy Bodies with α-Synuclein. Polyubiquitination of α-Synuclein is catalyzed by Nedd44, another ubiquitin ligase. Nedd44 adds ubiquitin chains to lysine residues, contributing to lysosomal degradation. Reversing ubiquitination in α-Synuclein is possible through the deubiquitinating enzyme USP9X. (Rott et al. 2017)

The effects of ubiquitination on α-Synuclein degradation are not fully understood. However, multiple studies show that this modification may play a regulatory role on α-Synuclein degradation. The effects of ubiquitination on α-Synuclein aggregation are also unclear. Some studies have shown that ubiquitination may have a protective role against α-Synuclein aggregation via proteasomal degradation. Ultimately, very little is known about ubiquitination and further research is needed to arrive at a definitive conclusion regarding its effects on α-Synuclein. (Engelander, 2008)

SUMOylation

SUMOylation is a reversible post-translational modification characterized by the addition of SUMO (small ubiquitin-related modifier) proteins to the lysine residues of target substrate proteins. SUMO proteins, like their name suggests, are closely related to ubiquitin. These proteins, when bonded with other proteins, can change their function. So far, four forms of the SUMO protein have been discovered in mammals. Much like ubiquitination, SUMOylation occurs due to an enzymatic cascade. However, unlike ubiquitination, SUMOylation is not used for the process of protein degradation. SUMOylation regulates lots of cell and protein functions, such as the cell cycle and transcription. SUMOylation can also alter protein localization, stability, and activity. SUMOylation occurs via a sequence of reactions. First, SUMO proteins go through C-terminal proteolytic cleavage, which exposes a glycine residue. This reaction is catalyzed by an enzyme called SAE1 (SUMO-activating enzyme E1). This exposed glycine residue can be attached to lysine residues in the target protein via the activity of an E2 enzyme called UBC9 (ubiquitin-conjugating enzyme 9). SUMOylation can be reversed through the activity of enzymes in a process called deSUMOylation. SUMOylation is an interesting protein modification that has many regulatory functions. Recent research has been shedding light on this modification, however there is much about SUMOylation to be understood so further research is needed. (Wilkinson et al. 2010)

SUMOylation may have regulatory effects on α-Synuclein, as well as effects on its aggregation. First, SUMOylated α-Synuclein may be able to regulate mitochondrial function. This could counteract abnormal mitochondrial function found in Parkinson's disease. SUMOylation can also form extracellular inclusions of α-Synuclein. Two enzymes are primarily associated with the SUMOylation of α-Synuclein. These enzymes are called PIAS and PIAS2, both of which are E3 enzymes. The effect of SUMOylation on α-Synuclein aggregation is widely disputed. In one study involving rat models, SUMOylation was found to inhibit α-Synuclein aggregation. (Krumova et al. 2011) Other studies say that SUMOylation promotes α-Synuclein aggregation by preventing degradation of the protein. When a lysine residue is ubiquitinated and SUMOylated, SUMOylation may interfere with the ubiquitination. This inhibits the degradation of α-Synuclein, which may lead to increased aggregation of the protein into Lewy Bodies. Further research is needed to fully understand the effect of SUMOylation on α-Synuclein, as there are a number of conflicting theories on the PTM's effects on α-Synuclein and its aggregation. (Rott et al. 2017)

Conclusion

Post-translational modifications are complex chemical processes that have a wide array of effects on proteins, cells, and diseases. Many of these post-translational modifications have been identified and research on PTMs is ongoing. Some of these post-translational modifications are correlated with the pathogenesis of Parkinson's

disease through α-Synuclein. α-Synuclein is a protein heavily associated with Parkinson's disease. The protein forms aggregates called Lewy Bodies which are linked to the pathology of Parkinson's disease. Post-translational modifications have a variety of effects on α-Synuclein and its properties, including its inclination to form aggregates. This implies that post-translational modifications of α-Synuclein play a crucial role in the pathogenesis of α-Synuclein, indicating that this is an important area of research. While there has been a growing body of research focusing on PTMs of α-Synuclein, more research is required to fully understand the intricacies of how α-Synuclein is affected by PTMs.

Table 1. PTMs of α-Synuclein, related enzymes, and effects. Created by Surya Kapu (2024, February 29)

PTM	Enzyme	Effect	References
Phosphorylation	GRK2	Promotes aggregation	Kawahata et al. 2022
	CKII		
	PLK2		
Acetylation	-	Resists aggregation	Bartels et al. 2014
Truncation	Calpain I	Promotes aggregation	Zhang et al. 2022
	Caspase I		
	Neurosin		
O-GlcNAcylation	OGT	Inhibits aggregation	Levine et al. 2019
Ubiquitination	SIAH	May inhibit aggregation	Engelander, 2008
	Nedd44		
SUMOylation	PIAS	May promote aggregation	Rott et al. 2017
	PIAS2		

There are some limitations in researching post-translational modifications of α-Synuclein. First, many studies focusing on PTMs are in vitro. The results of these studies may not translate to what actually happens in vivo. The studies that are in vivo are more accurate, however these are primarily performed in model organisms like mice and rats, not humans. Another limitation is the use of recombinant α-Synuclein. Recombinant α-Synuclein is artificially produced for laboratory research. The effects that PTMs have on recombinant α-Synuclein may not be the same as the effects they have on human α-Synuclein. Additionally. laboratory studies do not fully emulate all of the qualities and functions of PTMs. This means that there could be some other aspects of PTMs affecting α-Synuclein that are not fully taken into consideration. Finally, the interaction of α-Synuclein with other proteins, cells, and even the interactions between different PTMs is not always taken into account. More accurate forms of research can further elucidate the role that PTMs play in Parkinson's disease. PTMs are crucial processes that affect α-Synuclein, its aggregation, and ultimately the pathogenesis of Parkinson's disease. Research into PTMs is crucial, and could lead to novel therapies that help the millions of people that have been diagnosed with Parkinson's disease.

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References

"Statistics: Who has Parkinson's?" Parkinson's Foundation. Retrieved December 24, 2023, from https://www.parkinson.org/understanding-parkinsons/statistics

Stefanis L. (2011, December 11). *α-Synuclein in Parkinson's disease.* Cold Spring Harbor perspectives in medicine. Retrieved December 26, 2023, from https://doi.org/10.1101/cshperspect.a009399

Ramazi, S., Zahiri, J. (2021, April 7). *Post-translational modifications in proteins: resources, tools and prediction methods*. Database. Retrieved December 26, 2023, from https://doi.org/10.1093/database/baab012

Meade, R. M., Fairlie D. P., Mason, J. M. (2019, July 22). *Alpha-synuclein structure and Parkinson's disease – lessons and emerging principles*. Mol Neurodegeneration. Retrieved December 28, 2023, from https://doi.org/10.1186/s13024-019-0329-1

Bartels, T., Ahlstrom, L. S., Leftin, A., Kamp, F., Haass, C., Brown, M. F., & Beyer, K. (2010, October 6). *The N-terminus of the intrinsically disordered protein α-synuclein triggers membrane binding and helix folding*. Biophysical journal. Retrieved January 2, 2024, from https://doi.org/10.1016/j.bpj.2010.06.035

Xu, L., Nussinov, R., & Ma, B. (2016, October 4). *Coupling of the non-amyloid-component (NAC) domain and the KTK(E/Q)GV repeats stabilize the α-synuclein fibrils.* European journal of medicinal chemistry. Retrieved January 2, 2024, from https://doi.org/10.1016/j.ejmech.2016.01.044

Sorrentino, Z. A., Vijayaraghavan, N., Gorion, K., Riffe, C. J., Strang, K. H., Caldwell, J., Giasson, B. I. (2018, December 7). *Physiological C-terminal truncation of α-synuclein potentiates the prion-like formation of pathological inclusions.* J Biol Chem. Retrieved January 4, 2024, from https://doi.org/10.1074/jbc.RA118.005603

Kawahata, I., Finkelstein, D. I., & Fukunaga, K. (2022, June 1). *Pathogenic Impact of α-Synuclein Phosphorylation and Its Kinases in α-Synucleinopathies*. International journal of molecular sciences. Retrieved January 4, 2024, from https://doi.org/10.3390/ijms23116216

Xu, Y., Deng, Y. and Qing, H. (2015, July 2). *The phosphorylation of α-synuclein: development and implication for the mechanism and therapy of the Parkinson's disease.* J. Neurochem. Retrieved January 4, 2024, from https://doi.org/10.1111/jnc.13234

Chen, W. R., Chen, J. C., Chang, S. Y., Chao, C. T., Wu, Y. R., Chen, C. M., & Chou, C. (2022, December). *Phosphorylated α-synuclein in diluted human serum as a biomarker for Parkinson's disease.* Biomedical journal. Retrieved January 5, 2024, from https://doi.org/10.1016/j.bj.2021.12.010

Christensen, D. G., Xie, X., Basisty, N., Byrnes, J., McSweeney, S., Schilling, B., & Wolfe, A. J. (2019, July 12). *Post-translational Protein Acetylation: An Elegant Mechanism for Bacteria to Dynamically Regulate Metabolic Functions*. Frontiers in microbiology. Retrieved January 7, 2024, from https://doi.org/10.3389/fmicb.2019.01604

Xia, C., Tao, Y., Li, M., Che, T., & Qu, J. (2020, July 29). *Protein acetylation and deacetylation: An important regulatory modification in gene transcription (Review)*. Experimental and Therapeutic Medicine. Retrieved January 8, 2024, from https://doi.org/10.3892/etm.2020.9073

Choudhary, C., Kumar, C., Gnad, F., Nielsen, M. L., Rehman, M., Walther, T. C., Olsen, J. V., & Mann, M. (2009, July 16). *Lysine acetylation targets protein complexes and co-regulates major cellular functions.* Science. Retrieved January 8, 2024, from https://doi.org/10.1126/science.1175371

Bell, R., Vendruscolo M. (2021, July 14). *Modulation of the Interactions Between α-Synuclein and Lipid Membranes by Post-translational Modifications.* Centre for Misfolding Disease. Retrieved January 9, 2024, from https://doi.org/10.3389/fneur.2021.661117

Bartels, T., Kim, N. C., Luth, E. S., & Selkoe, D. J. (2014, July 30). *N-alpha-acetylation of α-synuclein increases its helical folding propensity, GM1 binding specificity and resistance to aggregation.* PloS one. Retrieved January 9, 2024, from https://doi.org/10.1371/journal.pone.0103727

Mueller, F., Friese, A., Pathe, C., da Silva, R. C., Rodriguez, K. B., Musacchio, A., & Bange, T. (2021, January 15). *Overlap of NatA and IAP substrates implicates N-terminal acetylation in protein stabilization*. Science advances. Retrieved January 11, 2024, from https://doi.org/10.1126/sciadv.abc8590

Fortelny, N., Pavlidis, P., & Overall, C. M. (2015, June 15). *The path of no return--Truncated protein N-termini and current ignorance of their genesis*. Proteomics. Retrieved January 11, 2024, from https://doi.org/10.1002/pmic.201500043

López-Otín, C., & Bond, J. S. (2008, November 7). *Proteases: multifunctional enzymes in life and disease.* The Journal of biological chemistry. Retrieved January 11, 2024, from https://doi.org/10.1074/jbc.R800035200

DeBoever, C., Tanigawa, Y., Lindholm, M.E. et al. (2018, April 24). *Medical relevance of protein-truncating variants across 337,205 individuals in the UK Biobank study.* Nat Commun. Retrieved January 12, 2024, from https://doi.org/10.1038/s41467-018-03910-9

Zhang, C., Pei, Y., Zhang, Z., Xu, L., Liu, X., Jiang, L., Pielak, G. J., Zhou, X., Liu, M., & Li, C. (2022, August 9). *C-terminal truncation modulates α-Synuclein's cytotoxicity and aggregation by promoting the interactions with membrane and chaperone*. Communications biology. Retrieved January 12, 2024, from https://doi.org/10.1038/s42003-022-03768-0

Dufty, B. M., Warner, L. R., Hou, S. T., Jiang, S. X., Gomez-Isla, T., Leenhouts, K. M., Oxford, J. T., Feany, M. B., Masliah, E., & Rohn, T. T. (2007, May). *Calpain-cleavage of alpha-synuclein: connecting proteolytic processing to disease-linked aggregation.* The American journal of pathology. Retrieved January 13, 2024, from https://doi.org/10.2353/ajpath.2007.061232

Mannino, M. P., & Hart, G. W. (2022, January 30). *The Beginner's Guide to O-GlcNAc: From Nutrient Sensitive Pathway Regulation to Its Impact on the Immune System.* Frontiers in immunology. Retrieved January 14, 2024, from https://doi.org/10.3389/fimmu.2022.828648

Levine, P. M., Galesic A., Balana A. T. et al. (2019, January 16). *α-Synuclein O-GlcNAcylation alters aggregation and toxicity, revealing certain residues as potential inhibitors of Parkinson's disease.* Proceedings of the National Academy of Sciences. Retrieved January 14, 2024, from https://doi.org/10.1073/pnas.1808845116

Guo HJ., Rahimi N., Tadi P.(2023 Mar 16). *Biochemistry, Ubiquitination.* StatPearls. Retrieved January 28, 2024, from https://www.ncbi.nlm.nih.gov/books/NBK556052/#:~:text=Ubiquitination%20is%20a%20tightly%20regulated,remove%20unwanted%20or%20damaged%20proteins.

Rott, R., Szargel, R., Shani, V., Hamza, H., Savyon, M., Abd Elghani, F., Bandopadhyay, R., & Engelender, S. (2017, November 27). *SUMOylation and ubiquitination reciprocally regulate α-synuclein degradation and pathological aggregation.* Proceedings of the National Academy of Sciences of the United States of America. Retrieved February 3, 2024, from https://doi.org/10.1073/pnas.1704351114

Engelender S. (2008, January 18). *Ubiquitination of alpha-synuclein and autophagy in Parkinson's disease. Autophagy.* Retrieved February 11, 2024, from https://doi.org/10.4161/auto.5604

Wilkinson, K. A., & Henley, J. M. (2010, May 13). *Mechanisms, regulation and consequences of protein SUMOylation.* The Biochemical journal. Retrieved February 13, 2024, from ttps://doi.org/10.1042/BJ20100158

Krumova, P., Meulmeester, E., Garrido, M., Tirard, M., Hsiao, H. H., Bossis, G., Urlaub, H., Zweckstetter, M., Kügler, S., Melchior, F., Bähr, M., & Weishaupt, J. H. (2011, July 11). *Sumoylation inhibits alpha-synuclein aggregation and toxicity*. The Journal of cell biology. Retrieved February 25, 2024, from https://doi.org/10.1083/jcb.201010117

Ramalingam, N., & Dettmer, U. (2023, November 13). *α-Synuclein serine129 phosphorylation - the physiology of pathology.* Molecular neurodegeneration. Retrieved February 29, 2024, from https://doi.org/10.1186/s13024-023-00680-x

Savyon, M., & Engelender, S. (2020, June 25). SUMOylation in α-Synuclein Homeostasis and Pathology. Frontiers in aging neuroscience. Retrieved February 29, 2024, from https://doi.org/10.3389/fnagi.2020.00167