Characterization of Familial Mutants and Splice Variants of Parkinson's Disease Protein α- Synuclein in Yeast Models

Natalie Kukulka
Department of Biology
Lake Forest College
Lake Forest, Illinois 60045

Abstract

The misfolding of the protein α-synuclein is a major contributor to Parkinson's disease (PD). Three mutations (A53T, A30P and E46K) cause familial PD, and three newly discovered spliced variant forms of the protein (syn-126, syn-112, and syn-98) are also found in many PD patients. Little is known about whether these familial mutants can influence each other's contributing properties and whether the spliced variants are protective or harmful. Each familial mutant distinctively affects α-synuclein's cellular localization, aggregation, and toxicity. For my thesis, I first tested the hypothesis that all three familial mutants equally influence a-synuclein's pathological contributions in yeast models and found unexpected support for the dominance of the A30P mutant over E46K and A53T, shedding new light on A30P's influence of a-synuclein's conformation. Using polymerase chain reaction-based strategies, I have also made significant progress in creating the three spliced-variants for future evaluation in yeasts to assess their contributions to PD.

Introduction

Nerve-Racking Protein Responsibilities

Understanding of the complexity of the human body is one of the most sought after abilities that doubtfully will ever be acquired. Take into consideration the human brain; it is a mass of about three pounds composed of one hundred billion nerve cells and trillions of supporting cells (Purves et al., 2012). This fundamental processing unit is responsible for everything one does, sees, smells, interacts with, has an emotional responses to or ignores altogether. Anatomically speaking, the brain is a part of the central nervous system, and along with the spine, both are responsible for integrating information and coordinating activity (Purves et al., 2012). In addition to this command system, the peripheral nervous system helps communicate with the rest of the body, thereby establishing a circuit of sensing, integrating, and acting.

As a newborn develops from an inexperienced 'creature' to a skilled adult, the range of processed information increases exponentially. Such overload of information is sequentially synchronized by the establishment of neuronal connections, which can either be retained with repetition or lost due to their irrelevance (Purves et al., 2012). On the molecular level, each neuron operates by an array of proteins whose functions are determined through their specific shapes. Although proteins are made up of as few as twenty amino acids, the sequence of the amino acid assortment is what leads to the diversity in protein shapes and consequent varied functions. While the protein folding machinery is quite robust, it operates best at an equilibrium state between environmental factors, cellular conditions, genetic influences, and foreign substances (Shin et al., 2009; Figure 1A). Increased exposure to negative environmental factors such as pollution or UV radiation or prolonged exposure to metals or toxins (such as pesticides) could contribute to the misfolding of the protein (Alberts et al., 2011). The molecular factors negatively influencing protein conformation could arise from incorrect encoding/ transcription of the DNA via various pathways or the failure of one of the intermolecular organelles like the mitochondria that serves as the power house for the cell (Shin et al., 2009). Still, more often than not, the brain withstands the negative factor of various sources and allows us to function flawlessly on a daily basis.

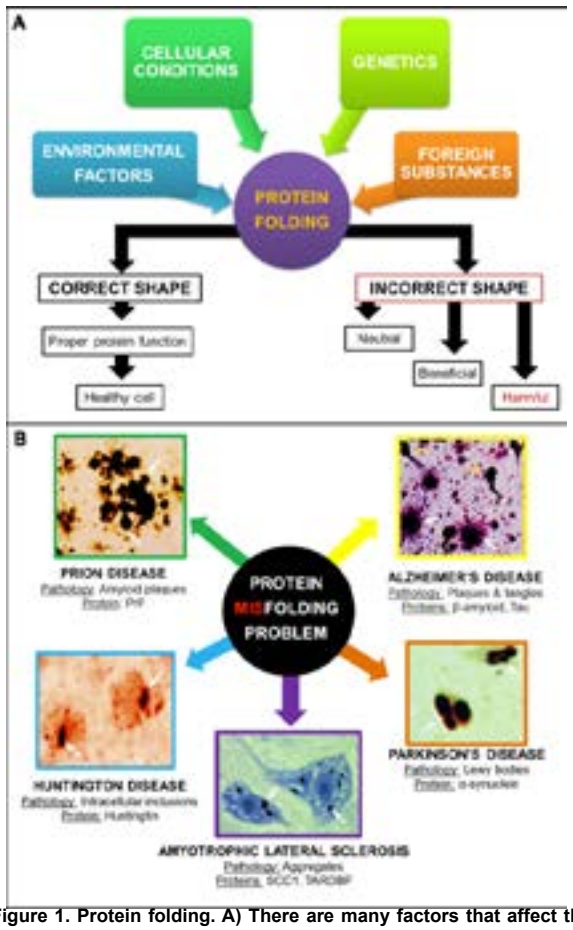

Figure 1. Protein folding. A) There are many factors that affect the folding process of the protein. Exhibited are just some of the factors that will influence protein conformation: environmental factors, cellular conditions, genetics, and exposure to foreign substances. If the environmental factors do not provide any harm to the organism, cellular conditions are appropriate, genetic information is not impaired, and exposure to foreign substances is minimal, then the protein will fold correctly. The proper conformation will then lead to a well-functioning protein and a healthy cell. However, if the balance between these factors is disrupted or they individually become unfavorable for the organism, then the protein may misfold. The altered shape will lead to neutral, beneficial, or harmful consequences. B) Neurodegenerative diseases are one branch of harmful consequences due to protein misfolding. Exemplified are five common neurodegenerative diseases along with a list of their pathological characteristics and the misfolded protein(s). The images for various neurodegenerative diseases were acquired from http://www.nature.com/nrn/journal/v4/n1/fig_tab/nrn1007_F1.html.

*This author wrote the paper as a part of a senior thesis under the direction of Dr. DebBurman

Neurodegeneration Initiated by Protein Misfolding

One of the negative consequences of improper protein folding can lead to selective death of neurons consequently leading to a neurodegenerative disorder (Figure 1B). Neurodegenerative diseases are disorders that are derived from the progressive deterioration of a specific portion of the nervous system, or in other words, a specific part of the brain that specializes in a particular ability (Ross & Poirier, 2004). Some of the leading neurodegenerative diseases are: Alzheimer's disease (AD), Parkinson's disease (PD), Huntington disease (HD), Multiple sclerosis (MS), amyotrophic lateral sclerosis or Lou Gehrig's disease (ALS), and Prion diseases (Figure 1B).
While each of these diseases differs in the progressive death of highly specialized neurons, the common feature among the pathology of neurodegenerative disease is the formation of protein aggregates from the misfolded protein in the affected neurons (Taylor et al., 2002; Figure 1B). The vast spectrum of neurodegenerative disease can be further narrowed down by focusing on the exact protein that misfolds. Synucleinopathies are thus neurodegenerative diseases in which the α-synuclein protein misfolds. They include dementia with Lewy bodies (DLB), multiple system atrophy (MSA), Lewy body dysphagia (LBD) and Parkinson's disease (Galvin & Trokanowscki, 2001). Despite years of extensive research, cures for any of these neurodegenerative diseases remain elusive, afflicting several regions of the brain. Due to the shared pathology, understanding the mechanisms of α-synuclein protein misfolding in one disease may unlock mysteries for the other synucleinopathies and neurodegenerative diseases. My thesis focuses on gaining insight into the molecular basis of α-synuclein that contributes to familial and sporadic PD. Nevertheless, my findings have the potential to help understand the basis of protein misfolding-linked problems in all synucleinopathies.

Understanding Parkinson's Disease

PD is the second most common neurodegenerative disease and the first most common bradykinesia disorder, known as the slowness of movement disorder (National Institute of Neurological Disorders and Stroke, 2004). While its occurrence was first noted by James Parkinson and documented in 1817 as a short article called "An Essay on Shaking Palsy", it continues to persist in even higher frequency two centuries later (Parkinson, 1817). PD afflicts close to ten million people worldwide and approximately 60,000 Americans annually (Parkinson's Disease Foundation, 2012). While the search for a cure for PD has broadened worldwide, so did the cost of possible treatments. With no possible cure on the horizon, patients try their luck with administering L-Dopa or using much more invasive treatment options, such as surgical implantation of metal electrodes for deep brain stimulation (Parkinson's Disease Foundation, 2012). While some of the symptoms may be initially suppressed, such as rigidity, resting tremors, masked facial expressions, patients ultimately die of organ failure (Olanow & Tatton, 1999; Galvin & Trokanowscki, 2001; Figure 2A).

PD Causes

PD can be classified as sporadic, which accounts for 90% of all diagnoses, or familial, which accounts for the remaining 10%. The sporadic form of PD can be initiated by various factors similar to those influencing protein misfolding (Figure 1A). For a long time, environmental factors were neglected in relation to the onset of PD, and it was not until a famous incident with the use of MPTP (1-Methyl-4-phenyl-1,2,5,6-tetrahydropyridine), a by-product of synthetic heroin, that people began pay closer attention (Sian et al., 1999). For example, exposure to chemicals like rotenone (an organic farming pesticide), (Betarbet et al.,

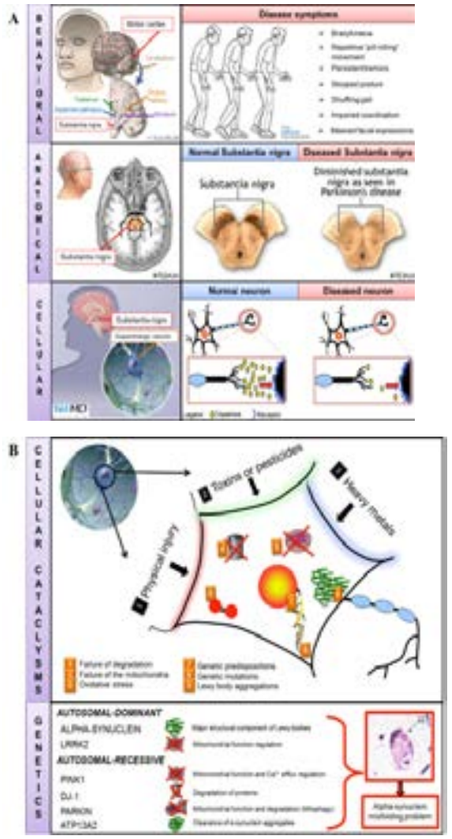

Figure 2. The basis of PD. A) Showcased are behavioral, anatomical, and cellular consequences of PD pathology demonstrated by restricted range of movement, neuronal death and reduced neurotransmitter signaling, respectively. The behavioral panel showcases the affected brain circuitry leading to the symptoms of the disease (image borrowed from https://www.dana.org/news/brainhealth/detail.aspx?id=9860). The anatomical panel shows the midbrain region of the substantia nigra, where at the onset of the disease, 90% of dopaminergic neurons die; this demise of neural cells can be clearly identified during patient autopsy through the lack of dark pigmentation (melanin) as exemplified on the right-side panel (image borrowed from http://www.umm.edu/patiented/articles/what_parkinsons_disease_what_causes_it_000051_1.htm). The cellular panel shows the release of the neurotransmitter, dopamine, from the specific dopaminergic neurons and how it diminishes during PD (image borrowed from http://www.webmd.com/parkinsons-disease/guide/parkinsons-causes). B) Showcased are molecular causes leading to the onset of the disease. The cellular cataclysms panel shows both the external influences on the neuron, as well as the molecular dysfunctions that lead to neurodegenration, especially in regard to PD. The genetics panel shows different genes that result in specific molecular consequences leading to the formation of Lewy bodies.

2000), heavy metals (Calne et al., 1994), and free radicals (Maguire-Zeiss et al., 2005) have been linked with sporadic PD (Figure 2B).

Another factor, classified as a long-term environmental influence, was linked with traumatic brain injury, such as a traumatic blow to the head or a severe concussion. In terms of cellular dysfunction, oxidative stress (Jenner & Olanow, 1996; Maguire-Zeiss et al., 2005) and mitochondrial incapability (Langston et al., 1983; Langston et al., 1984) have been tied into PD (Figure 2B). Nevertheless, the precise mechanisms behind these factors are not well known, hence researchers tend to focus on the genetic factors within the familial branch of PD. The most common gene mutations that lead to PD occur in SNCA (Polymeropoulos et al., 1997; Kruger et al., 1998; Zarranz et al., 2004), Parkin Kidata et al., 1998), UCH-L1 (Liu et al., 2002), DJ-1 (Bonifati et al., 2003), PINK1 (Valente et al., 2004) and LRRK2/PARK8 (Funayama et al., 2002; Paisan-Ruiz et al., 2004, Liu et al., 2012), PARK2, PARK7 (Neuytemans et al., 2010) autosomal-dominant PD mutations in (UCHL-1, SNCA, LRRK2), and autosomal-recessive PD (parkin, PINK1 and DJ-1; Figure 2B).

Parkinson's Disease Pathology

PD is characterized by the death of dopaminergic neurons predominately concentrated within the midbrain structure known as the substantia nigra, which is Latin for "black substance" (Figure 2A). The substantia nigra is critical in the circuitry known to govern reward, addiction, and movement, which is further consistent with the exhibited symptomatic death of these neurons (Figure 2A). PD pathology reveals protein aggregates within degenerating neurons, which Frederick Lewy first named Lewy bodies in 1912. It was not until 1997 that Polymeropoulos and colleagues found a mutation in the α-synuclein gene, SNCA, on the fourth human chromosome, that caused autosomal-dominant PD through the A53T mutation in the α-synuclein protein (Polymeropoulos, 1997). The identified misfolded and aggregated α-synuclein protein was thus recognized as the major component that makes up Lewy bodies (Spillantini et al., 1998). Recently, it has been shown that Lewy bodies are formed from both full length and truncated versions of α-synuclein, indicating that the balance between these isomers plays an important role in the onset and progression of the disease (McLean et al., 2102).

Insight into α-Synuclein

The synuclein protein was first isolated from Torpedo californica, or Pacific electric ray, but now its complete sequence has been analyzed in more than 20 species with its respective mutations, posttranscriptional modifiers, polymorphisms and truncations (Xiong et al., 2010). While α-synuclein is predominantly expressed in the central nervous system, it is not a brain-specific protein because its presence is recognized in other tissues, such as the heart, muscles and pancreas (Beyer et al., 2004, Beyer et al., 2006). Further research has specified and confirmed its high expression in skin, lungs, kidney, spleen, heart, liver and muscle samples (Beyer et al., 2008). Still, its highest expression is found in the brain (Beyer et al., 2008).

Structurally speaking, α-synuclein is a small, 140-amino-acid-long acidic protein, which is encoded by the SNCA gene on chromosome 4q21 (Beyer et al., 2006). Its native unfolded form increases its predisposition to self-aggregate based on varying environmental conditions, making it a very dynamic molecule (Lucking & Brice, 2000, Beyer et al., 2006, Bisaglia et al., 2009). More specifically, in an aqueous solution, it conserves its unfolded and randomly coiled structure. However, when it comes in contact with acidic phospholipid vesicles, it folds into an α-helical structure or forms insoluble fibrils with a high beta-sheet structure leading to formation of Lewy bodies (Beyer et al., 2006, Beyer et al., 2008).

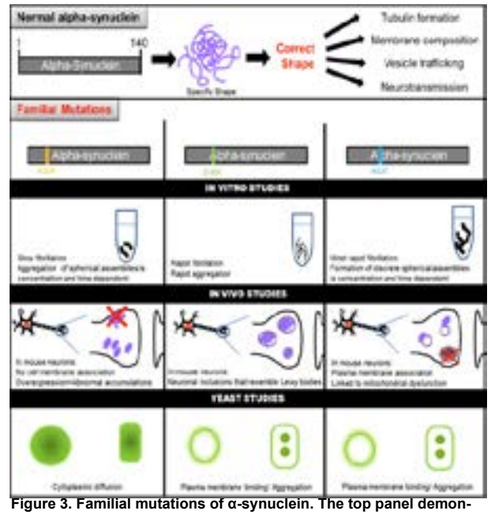

Figure 3. Familial mutations of α-synuclein. The top panel demonstrates the roles of the properly folded and corrently functioning α-synuclein prtein. This description is then contrasted by the portrayal of consequences from each of the familal mutations: A30P, E46K, and A53T in vitro, in vivo, and in yeast studies.

Aside from environmental conditions, there are genetic factors that may alter the characteristics of a protein. Three distinct α-synuclein single amino acid point mutations lead to the development of familial PD. The data in my thesis depends heavily upon the understanding of each individual mutant's characteristics. Therefore, I will elaborate on them in the overview for study one. In additional genetic mutations, there are more than three hundred different posttranslational protein modifications which lead to changes in protein size, charge, structure, and conformation, affecting key protein characteristics. As part of background information for study two, I will provide insight into understanding one of these posttranslational protein modifications: the formation of splice variants.

STUDY 1

Understanding Familial PD Point Mutations

A53T:

This missense mutation, where alanine charges to threonine on the 53rd amino acid of the α-synuclein protein, was the first familial PD point mutation identified in the Contursi family and three additional unrelated Greek families (Polymeropoukous et al., 1997; Figure 3). In in vitro studies, the A53T mutant showed that in the absence of other Lewy body-associated molecules, it was disordered in dilute solution just as WT, but at high concentrations it formed discrete spherical assemblies at the fastest rate (Conway et al., 1998; Figure 3). Further in vitro experimentation supported A53T's ability to aggregate best at lower concentrations and showed that A53T had greater propensity to polymerize than A30P, one of the other familial mutants (Giasson et al., 1998). In 2000, Conway et al.'s research findings added that the fibrillation of A53T was relative to that of WT, and faster than that of A30P, while the consumption of the A53T monomer was the most rapid.

In 2001, the study conducted by Sharon et al. not only shed more light into the properties of the A53T α-synuclein mutation, but also into the function of the α-synuclein protein itself. In their transgenic mouse model, they found an enhanced association of the A53T mutation with microsomal membranes consequently leading to the conclusion that α-synuclein is a novel member of the lipid-binding protein family (Sharon et al., 2003; Figure 3). Further spectroscopy analysis showed no significant difference from WT in the second structure upon membrane binding of α-synuclein (Jo et al., 2002). Studies in living organisms, more precisely in yeast models, revealed that in budding yeast the A53T mutation showed plasma membrane binding, just like WT, and in fission yeast it was exhibited as accumulation of small vesicles or the presence of aggregates (Sharma et al., 2006, Brandis et al., 2006; Figure 3). Depending on the strain that was being used as the model, A53T did not show any toxicity in budding yeast, yet in fission yeast it was always toxic in either the TCP1 or Sph+ strains. (Sharma et al., 2006, Brandis et al., 2006; Figure 3).

In respect to human dopamine transporter (hDAT) the mutation did not form strong protein-to-protein bonds, and unlike A30P, it did not modulate dopamine uptake from hDAT in any way (Wersinger et al., 2003). Nevertheless, the hDAT-dependent toxicity was higher than with either WT or A30P (Wersinger et al., 2003). In transgenic mice models, the A53T mutant of α-synuclein led to development of severe and complex motor impairment, leading to paralysis and death (Giasson et al., 2002). Further immunoelectron microscopies supported the argument that the extensively formed filaments were toxic and lead to neurodegeneration (Giasson et al., 2002). The latest research further elaborates upon A53T's effects and shows that the overexpression of the mutant leads to massive mitochondrial destruction and loss, which is a bioenergetics deficit characteristic of neural degeneration (Choubey et al., 2011).

A30P:

This missense mutation was the second point mutation identified in the involvement of the onset of familial PD after A53T. In 1998, members of a British family lacking the A53T mutation revealed a hereditary progression of the development of PD. After further investigation, Kruger at al. identified the A30P missense mutation (a switch of Alanine to Proline on the 30th amino acid in the α-synuclein) as a familial PD point mutation in accordance with the UK Parkinson's Disease Society Brain Bank's criteria (Figure 3).

When tested in a vesicle binding assay, the A30P mutation lacked significant plasma membrane binding, which was initially proposed to lead to the assembly of Lewy body filaments in the rat optic system (Jensen et al., 1998; Figure 3). Subsequent in vitro studies showed that in the absence of other Lewy body-associated molecules, the A30P α-synuclein mutant was disordered in dilute solution, just as WT, whereas at high concentrations it only formed small spherical species (Conway et al. 1998). Further in vitro experimentations showed that A30P demonstrated an ability to aggregate, which depended on both concentration and time (Giasson et al., 1998). In 2000, Conway et al.'s research findings explained that although the fibrillation of A30P was slower than that of WT and A53T, the consumption of the A30P monomer was comparable to or slightly faster than WT, but slower than the rate of A53T. Such findings could be attributed to the fact that unlike other Parkinson's disease mutations, A30P has been shown to substantially alter the three-dimensional conformation of the α-synuclein protein, which may be due to the failure to undergo a typical structural transition from random coil to α-helix (McLean et al., 2000, Jo et al., 2002).

Later studies in living organisms, for instance in yeast models, revealed that in both budding and fission yeast the A30P mutant exhibited cytoplasmic diffusion throughout the cell and was not toxic in either of the models (Sharma et al., 2006, Brandis et al., 2006; Figure 3). In 2000, Kahle et al. demonstrated that the A30P human α-synuclein mutant in a transgenic mouse's brain did not fail to be transported to the synapse, despite its inhibited ability to bind plasma membranes. In fact, A30P's transgenic overexpression lead to abnormal cellular accumulations (Kahle et al., 2000). In relation to human dopamine transporter (hDAT), the mutation did form strong protein-to-protein complexes, and unlike A53T, it negatively modulated hDAT function, leading to reduced uptake of the extracellular dopamine and dopamine-mediated hDAT-dependent toxicity (Wersinger et al., 2003).

E46K:

In 2004, E46K was identified as the third familial PD point mutation in α-synuclein leading to familial PD (Zarranz et al., 2004). In E46K, the dicarboxylic amino acid—the glutamic acid—changes to lysine on the 46th position (Figure 3). Published by Zarranz et al. in 2004 as the first instance of its identification in a Spanish family, the mutation pathologically contributed to the death of dopaminergic neurons within the substantia nigra and presence of numerous Lewy bodies, which are key trademarks of PD. Additionally, the aggregations of the protein proved to be unresponsive to ubiquitin in cortical and subcortical areas, further supporting its role in the onset of PD (Zarranz et al., 2004). That same year, this missense mutation was characterized in terms of its phospholipid properties and ability to assemble filaments in comparison to the WT α-synuclein and the other two familial mutations, A30P and A53T. It turns out that E46K caused two fold higher proportion of liposomal binding and resulted in the same or slightly less high rate of formation of fibrils as A53T(Choi et al. 2004, Greenbaum et al., 2004; Figure 3).

Shortly after its identification, E46K was biochemically and biophysically characterized, revealing that it resulted from rather subtle changes to the conformation of the α-synuclein protein (Fredenburg et al., 2007). When tested in vitro, E46K formed insoluble fibrils more rapidly than WT but the total amount of protofibrils was reduced (Fredenburg et al., 2007). Using high-resolution atomic force microscopy, researchers established that structurally, E46K formed fibrillar aggregates of a smaller diameter and periodicity than WT (Raaij et al., 2006). At the same time, E46K yielded a larger amount (4.4-fold) of total accumulation and assembly of amyloid fibrils than WT at 25°C (Kamiyoshihara et al., 2007).

In culture systems, such as human neuroblastoma cells, E46K exhibited highest percentage of aggregation (in comparison to WT, A30P, and A53T), as well as phenotypic characteristics reminiscent of the formation of Lewy bodies (Pandey et al., 2005). At the same time, when E46K was tested in a live organism, budding yeast, it revealed a major lag in cell growth in the optical density assay, but those findings were contradicted by the toxicity results of the spotting assay that did not reveal any noticeable change in the rate of growth with the E46K mutation (Herrera & Shrestha, 2005; Figure 3). In transgenic mice, E46K human α-synuclein mutation expressed in SNpc resulted in neuronal inclusions that reiterated the biochemical, histological and morphological characteristics of Lewy bodies (Emmer et al., 2011).

Combinatory Mutations until Now

Although the knowledge on the individual familial PD mutations (A30P, E46K, and A53T) is vast and specific in areas such as in vitro, cell cultures, model organisms and human populations, there is little to no information in the literature

about any combinatory mutations. Certainly none of the human patients has been found to exemplify more than one familial mutation at the time, but due to the varied properties exemplified by each mutant, combining them would provide a novel insight into the structural dynamics of the α-synuclein protein. The earliest recognition of the significance of studying combinatory missense mutations appears in the literature in 2002 when Jo et al., characterize the A30P-A53T mutant in an in vitro study. Their results showed a defective membrane binding which mimicked that of A30P, therefore suggesting dominance of the proline mutation in regard to lipid membrane binding behavior (Jo et al., 2002, Fuller et al., 2002). Furthermore, these results were supported and further elaborated upon in experiments in budding and fission yeast. In both budding and fission yeast, the A30P-A53T α-synuclein mutation showed cytoplasmic diffusion throughout the cell (Sharma et al., 2006, Brandis et al., 2006). In budding yeast, the combinatory mutant appeared not be toxic, and in fission yeast, the A30P-A53T mutant was less toxic than either one of the individual mutations (Sharma et al., 2006, Brandis et al., 2006).

The earliest animal (rodent) model bearing any one of the combinatorial mutations was described by Ikeda et al., in 2008 where the researchers created TgαSYN transgenic mice to study the A30P-A53T mutation. Overall, within the striatum they found significantly decreased levels of dopamine and the rotarod test revealed motor impairment making their model useful for analyzing the pathological cascade (Ikeda et al., 2008). Later on in 2011, Lelan et al. used a transgenic Sprague Dawley rat to gather a better insight of the properties of the A30P-A53T α-synuclein mutation. The researchers hoped to gather further insight into neuropathological intricacies of PD. They found that the rat solely displayed olfactory deficits without any motor impairment which would be characteristic of an early onset PD, making it a good model to test olfactory deficits. While there was no α-synuclein aggregation localized in the subventricular zone, there was cell proliferation in the glomerular layers of the olfactory bulb and the subventricular zone (Lelan et al., 2011).

Gap in Knowledge

Despite minimal research available for A30P-A53T combinatory mutant, there is no literature on the other combinatory mutations, such as A30P-E46K, E46K-A53T or A30P-E46K-A53T. This lack of any sign of experimental analysis needs to be fulfilled, allowing for better understanding of the significance of these specific amino acids within α-synuclein.

STUDY TWO:

Molecular research focused on uncovering a specific set of protein properties can be initiated in two ways. The first option is to create synthetic variants that mimic one or more of the post-transitional modifications that the protein could be exposed to. The second technique is to recreate natural protein variants in a laboratory setting, which would allow for better control over protein expression leading to more specific insight.

Synthetic Variants

Three examples of post-transitional modification of α-synuclein that can be studied through designer mutants are phosphorylation, nitrosylation and sumoylation. LBs are known to be composed of highly phosphorylated α-synuclein, particularly at two serine locations: amino acid 87 and 129 (Fujiwara et al., 2002, Paleolegou et al., 2010). The kinase involved in the α-synuclein phosphorylation is yet to be identified; nevertheless, simulating this condition can bring further insight into PD pathology and possible kinases identification.

In regard to nitrosylation, there are four sites that have been linked to PD, which can be studied through four mutation mimics: T39K, T125K, T133K, T136K (Clayton & George, 1998). One of the more interesting findings from 2004, done by Hodara et al., showed that a nitration of Tyrosine 39 hindered the protein's ability to bind lipids and caused it to behave like the familial A30P mutation.

The process of sumoylation was recently linked to a novel family of proteins called SUMO that is believed to have the capacity to influence inter- and molecular interactions of α-synuclein. This modification could potentially prevent the protein from binding to itself, consequently reducing aggregation (Geiss-Friedlaner & Melchoir, 2007). Specifically, SUMO has been shown to modify lysine residues on proteins through establishing covalent bonds of which the most prominent two are K96 and K102 (Wilkinson et al., 2010, Bossis & Melchoir, 2006). Designer mutants such as K96R and K102R could therefore bring valuable insight into the role of this protein family.

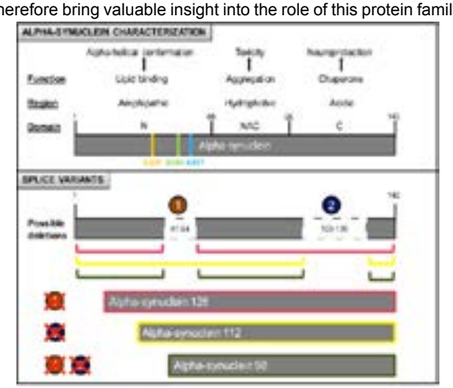

Figure 4. Natural variants of alpha-synuclein protein. The top panel (Alpha-Synuclein Characterization) breaks down the structural topography of the α-synuclein protein and provides the functions that each region is believed to be responsible for. The second panel (Splice Variants) shows the formation of natural splice variants through alternative splicing of α-synuclein gene, SNCA.

Natural Variants

Aside from post-translational modifications, there are other changes that may lead to formation of new protein variants such as polymorphisms and truncations of the protein. Protein polymorphisms occur when different genetic variants (alleles) become expressed. In one of the initial studies from 2003, conducted by Holzmann and his colleagues, two polymorphisms (-116C>G and -668T>C) of the α-synuclein promoter defining four haplotypes have been characterized in 315 German PD patients. In a later study from 2004, which focused on the Norwegian population, Myhre et al. identified several markers associated with PD such as Rep1 263 base pare allele, rs356165 and rs356219. In the Korean population the MAPT H1 haplotype and SNCA single nucleotide polymorphism (SNP) rs356219 have been reported to have a synergistic effect on the risk of PD (Kim et al., 2010). In regard to protein truncations, it has been established that about 15% of α-synuclein structures are truncated forms, which in vitro show high tendency for aggregation (Liu et al., 2005, Beyer & Ariza, 2012). Most specifically, the known forms of truncations are due to 20 S proteasome-mediated cleavage (Li et al., 2005), calpain-I-mediated cleavage (Dufty et al., 2007) and matrix metalloproteinase 3 mediated cleavage (Beyer & Ariza, 2012). Additionally, recently three naturally occurring splice variants have been identified resulting in the formation of α-synuclein-112, -126 and -98 (McLean et al., 2012).

Introduction to α-synuclein splice variants

The initial notion of the presence of splice variants of the α-synuclein protein was documented in 2004 in the study conducted by Beyer et al. The researchers identified an α-synuclein splice variant that was 112 amino acids long and was missing the region that accounted for the fifth exon (that was eliminated during alternative splicing), consequently deleting the amino acids from position 103 to 130 (Beyer et al., 2004; Figure 4). This alteration deletes the possible phosphorylation site at position 129, which plays an important role in accumulation (Xiong et al., 2010).

Furthermore, this truncation is highly hydrophobic because it is missing the majority of its hydrophilic amino acids located in exon five in this acidic C domain (Xiong et al., 2010).

In 2004, Beyer and his collogues compared the α-synuclein protein abundance levels of the full length version (140 amino acids long) and the spliced variant (α-synuclein-112) in human patients. They chose to analysis the prefrontal cortices of six patients with dementia with

Lewy bodies (DLB), eight patients with Alzheimer's disease (AD) and six control subjects. They found that the level of α-synuclein-112 was greatly elevated in patients with DLB as opposed to AD, while the levels of alpha-synuclein 140 were diminished in both cases (Beyer et al. 2004).

Later on, another splice variant was identified, α-synuclein126, which is caused by the absence of the 3rd exon (amino acids: 41-53) due to alternative splicing (Figure 4). These splice variant results in the deletion of two familial PD mutations, E46K and A53T, and based on the location of the deletion, it affects the α-helix structures at the N-terminus (Xiong et al., 2010). In 2006, Beyer et al. characterized α-synuclein-126 from patient deceased from DLB, AD, and controls. They found that in all cases, α-synuclein-126 levels were markedly diminished in comparison to that of α-synuclein-140 (Beyer et al., 2006).

Finally, in 2007, Beyer at al. identified the third splice variant called α-synuclein-98 (Figure 4). Due to alternative splicing, this protein lacks both exons 3 and 5, resulting in missing amino acids in the region from 41-53 and 103-130. Aside from the fact that this truncation is missing familial point mutations E46K and A53T, as well as the phosphorylation site at S129, its function is not yet fully understood (Xiong et al., 2010). Nevertheless, mRNA expression analyses revealed that α-synuclein-98 is a brain-specific splice variant and its expression varies based on location in fetal and adult brains (Beyer et al., 2008).

In the most recent study on α-synuclein splice variants published in 2012, McLean et al. analyzed both human brain regions and conducted studies on transgenic mice. In PD patients, they found that the truncations were expressed specifically in the cortex, substantia nigra, and cerebellum (McLean et al., 2012). The results in human α-synuclein expressing mice (ASO) revealed that while most transcript levels were elevated in comparison to WT mice, the highest levels were in the midbrain in 15-month old ASO mice (McLean et al., 2012).

Gap in Knowledge

Due to the recent identification of three α-synuclein splice variants, there is an absence of studies where each of the spliced variants would be individually expressed in any research model. Thus far, α-synuclein-126, -112, and -98 have only been examined during simultaneous expression in a model organism. Therefore, the gap in knowledge comes in understanding the function of each splice variant and later on the dynamics of their interactions.

MY EFFORTS

Earlier, I identified two gaps of knowledge that currently exist in understanding the PD protein α-synuclein. In order to narrow down the first presented gap of knowledge, I decided to create and assess α-synuclein combinatory mutants in several yeast models. In order to bridge the second gap, I set out to create at least two of the three splice variants, α-synuclein-126 and -112. The evidence mounted for this first study is compiled into the first chapter of my thesis, while the data gathered for the second study is compiled into the second chapter of my thesis.

The overall goal of my thesis was to gather further insight into the properties of α-synuclein and its relation to PD pathology. I attained that goal through: 1) assessing combinatory mutants in yeast models that allowed for the analysis of the α-synuclein's two hallmark PD related pathology characteristics, 2) creating tools for future assessment of the roles of each splice variant and their individual contribution to LB formation.

STUDY ONE:

Hypothesis and Aims

I hypothesized that combinatory mutants would exhibit: a blend of phenotypic features, high toxicity, and high protein accumulation (Figure 5). To test this hypothesis, I first created α-synuclein combinatory mutants: A30P-A53T, A30P-E46K, E46K-A53T, and A30P-E46K-A53T. Then, I assessed their properties in regard to α-synuclein localization, toxicity, and expression in several yeast models (Table 1).

Main Findings

Combinatory mutants were successfully created and expressed in low and high level protein expression yeast models. In terms of protein localization, A30P dominated the phenotype in combinatory variants. Furthermore, combinatory mutants did not enhance toxicity or protein expression.

STUDY TWO:

Hypothesis and Aims

I hypothesize that alpha-synuclein-98 and -126 will show diffusion throughout the cell, due to the impairment of regions responsible for plasma membrane binding; while α-synuclein-112 will show the formation of aggregates. Furthermore, I hypothesize that α-synuclein-112 and -98 will be more toxic than α-synuclein-126. Based on results from patients' data, I hypothesize that in yeast the protein expression will be the highest for α-synuclein-126, followed by -98, and finally -112. In order to test my hypothesis, I aimed to create tools for future assessment of each splice variant's role and contribution to PD pathology. My first goal was to create α-synuclein-126 and -112.

Main Findings

α-synuclein-126 and -112 were created through PCR splicing and α-synuclein-126 was successfully TOPO cloned.. Although none of the created α-synuclein splice variants have been sequenced, the α-synuclein-126 and -112 both seem to be of the appropriate sizes.

References

Alberts, B., Bray, D., Hopkin, K., Johnson, A., Lewis, J., Raff, M., …Walter, P. (2010). Essential Cell Biology. Garland Science: New York.

Benkert, P., Tosatto, S. C. E., & Schomburg, D. (2008). QMEAN: A comprehensive scoring function for model quality assessment. Proteins: Structure, Function, and Bioinformatics, 71, 261-277.

Betarbet, R., Sherer, T., MacKenzie, G., Garcia-Osuna, M., Panov, A., & Greenamyr, J. (2000). Chronic systemic pesticide exposure reproduces features of Parkinson's disease. Natural Neuroscience, 3, 1301-1306.

Beyer, K., Domingo-Sabat, M., Humbert, J., Carrato, C., Ferrer, I., & Ariza, A. (2008). Differential expression of alpha-synuclein, parkin, and synphilin-1 isoforms in Lewy body disease. Neurogenetics, 9, 163-172.

Beyer, K., Lao, J. I., Carrato, C., Mate, J. L., Lopez D., Ferrer, I., & Ariza, A. (2004). Differential expression of alpha-synuclein isoforms in dementia with Lewy bodies. Neuropathology and Applied Neurobiology, 30, 601-607.

Beyer, K. (2006). Review: Alpha-synuclein structure, posttranslational modification and alternative splicing as aggregation enhancers. Acta Neuropathologica, 112, 237-251.

Beyer, K., Humbert, J., Ferrer, A., Lao, J. I., Carrato, C., López, D., Ferrer, I., & Ariza, A. (2006). Low alpha-synuclein 126 mRNA levels in dementia with Lewy bodies and Alzheimer disease. Neuroreport, 17, 1327-1330.

Beyer, K., Domingo-Sabat, M., Lao, J. I., Carrato, C., Ferrer, I., & Ariza, A. (2008). Identification and characterization of a new alpha-synuclein isoform and its role in Lewy body diseases. Neurogenetics, 9, 15-23.

Beyer, K., & Ariza, A. (2012). Alpha-synuclein posttranslational modification and alternative splicing as a trigger for neurodegeneration. Molecular Neurobiology. Publlished online. DOI 10.1007/s12035-8330-5.

Bisaglia, M., Mammi, S., & Bubacco, L., (2009). Review: Structural insight physiological functions and pathological effects of alpha-synuclein. The Federation of American Society for Experimental Biology Journal, 23, 329-340.

Bonifati, V., Rizzu, P., van Baren, M. J., Schaap, O., Breedveld, G.J., Krieger, E., …. Heutink, P. (2003). Mutations in the DJ-1 gene associated with autosomal recessive early-onset parkinsonism. Science, 299, 256-259.

Bossis, G., & Melchoir, F. (2006). Regulation of SUMOylation by reversible oxidation of SUMO conjugating enzymes. Molecular Cell, 21, 349-357.

Brandis, K. A., Holmes, I. F., England, S. J., Sharma, N., Kukreja, L., & DebBurman, S. K. (2006). α-Synuclein fission yeast model concentration–dependent aggregation without plasma membrane localization or toxicity. Journal of Molecular Neuroscience, 28, 179-191.

Calne, D. B., Chu, N. S., Huang, C. C., Lu, C. S., & Olanow, W. (1994). Manganism and idiopathic parkinsonism: similarities and differences. Neurology, 44, 1583-1586.

Chan, S. C., Guzman, J. N., Ilijic, E., Mercer, J. N, Rick. C., Tkatch, T., … Surmeier, D.J. (2007). Rejuvenation protects neurons in mouse models of Parkinson's disease. Nature, 447, 1081-1086.

Choi, W., Zibaee, S., Jakes, R., Serpell, L., Davletov, B., Crowther, R.A., & Goedert, M. (2004). Mutation E64K increases phospholipid binding and assembly into filaments of human α-synuclein. Federation of the Societies of Biochemistry and Molecular Biology Letters, 576, 363-368

Choubey, W., Safiulina, D., Vaarmann, A., Cagalines, M., Wareski, P., Kuum, M., … Kaasik, A. (2011). Mutant A53T α-synuclein induces neuronal death by increasing mitochondrial autophagy. Journal of Biological Chemistry, 286, 1-18.

Clayton, D., & George, M. (1998). The synucleins: a family of proteins involved in synaptic function, plasticity, neurodegeneration and disease. Trends in Neuroscience, 21, 249-254.

Conway, K. A., Harper, J. D., & Lansbury Jr., P. T. (1998). Accelerated in vitro fibril formation by a mutant α-synuclein linked to early-onset Parkinson disease. Nature Medicine, 4, 1318-1320.

Conway, K. A., Lee, S. J., Rochet, J. C., Ding, T. T., Williamson R. E., & Lansbury Jr., P. T (2000). Acceleration of oligomerization, not fibrillization, is a shared property of both α-synuclein mutations linked to early-onset Parkinson's disease: Implications for pathogenesis and therapy. Proceedings of the National Academy of Sciences, 97, 571-576.

Dufty, B. M., Warner, L. R., Hou, S. T., Jiang, S. X., Gomez-Isla, T., Leenhouts, K. M., … Rohn, T. T. (2007). Calpain-cleavage of alpha-synuclein: connecting preteolytic processing to disease-linked aggregation. The American Journal of Pathology, 170, 1725-1738.

Fuller, N., Rand, R. P., George-Hyslop, S., & Fraser, P. E. (2002). Defective membrane interactions of familial Parkinson's disease mutant A30P alpha-synuclein. Journal of Molecular Biology, 315, 799-807.

Emmer, K. L., Waxman, E.A., Covy, J.P., & Giasson, B.I. (2011). E46K human α-synuclein transgenic mice develop Lewy-like and Tau pathology associated with age-dependent, detrimental motor impairment. Journal of Biological Chemistry, 286, 1-22.

Fiske M., Valtierra, S., Solvang, K., Zorniak, M., White, M., Herrera, S., Konnikova, A., Brezinsky, R., DebBurman, S. (2011), Contribution of alanine-76 and serine phosphorylation in alpha-synuclein membrane association and aggregation in yeasts, Parkinson's Disease, DOI: 10.4061/2011/392180

Fredenburg, R. A., Rospigliosi, C., Meray, R. K., Kessler, J. C.,

Lashuel, H. A., Eliezer, D., & Lansubry Jr., P.T. (2007). The impact of the E46K mutation on the properties of α-synuclein in its monomeric and oligomeric states. Biochemistry, 1-14.

Fujiwara, H., Hasegawa, M., Dohmae, N., Kawashima, A., Masliah, E., Goldberg, M. … Iwatsubo, T. (2006). Alpha-synuclein is phosphorylated in synucleinopathy lesions. Natural Cell Biology, 4, 160-164.

Funayama, M., Hasegawa, K., Kowa, H., Saito, M., Tsuji, S., & Obata, F. (2002). A new locus for Parkinson's disease (PARK8) maps to chromosome 12p11.2-q13.1. Annals of Neurology, 51, 296-301.

Geiss-Friedlander, R., & Melchoir, F. (2007). Concepts in sumoylation: a decade on. Nature Reviews Molecular Cell Biology, 8, 947-956.

Glavin, J., Lee, V., & Trojanowski, J. (2001) Synucleiopathies: clinical and pathological implications, Archives of Neurology, 58, 186-190

Giasson, B. I., Duda, J. E., Quinn, S. M., Zhang, B., Trojanowski, J. Q., & Lee, V. M. Y. (2002). Neuronal α-synucleinpathy with severe movement disorder in mice expressing A53T human α-synuclein. Neuron, 34, 521-533.

Giasson, B. I., Uryu, K., Trojanowski, J. Q., & Lee, V. M. Y. (1999). Mutant and wild type human α-synuclein assemble into elongated filaments with distinct morphologies in vitro. The Journal of Biological Chemistry, 274, 7619-7622.

Greenbaum, E. A., Graves, C. L., Mishizen-Elberz, A. J., Lupoli, M. A., Lynch, D. R., Englander, W., … Giasson, B. I. (2005). The E46K mutation in α-synuclein increases amyloid fibril formation. The Journal of Biological Chemistry, 250, 7800-7807.

Gromiha, M. (2003). Importance of native-state topology for determining the folding rate of two-state proteins. Journal of Chemical Information and Computer Sciences, 43, 1481-1485.

Herrera, S., & Shrestha, R. (2005). Newly discovered α-synuclein familial mutant E46K and key phosphorylation and nitrosylation-deficient mutants are toxic to yeast. Eukaryon, 1, 95-101.

Holzmann, C., Kruger, R., Saecker, A.M., Schmitt, I., Schols, L., Berger, K., & Riess, O. (2003). Polymorphisms of the α-synuclein promoter: expression analyses and association studies in Parkinson's disease. Journal of Neural Transmission, 110, 67-76.

Hodara, R., Norris, E., Giasson, B., Mishizen-Eberz, A., Lynch, D., Lee, M., & Ischiropoulos H. (2004). Functional consequences of alpha-synuclein tyrosine nitration: diminished binding to lipid vesicles and increased fibril formation. The Journal of Biological Chemistry, 279, 47746–47753.

Ikeda, M., Kawarabayashi, T., Harigaya, Y., Sasaki, A., Yama, S., Matsubara, E., … Shoji, M. (2009). Brain Research, 232-241.

Jenner, P., & Olanow, C. (1996). Oxidative stress and the pathogenesis of Parkinson's disease: a review. Neurology 47, S161-170.

Jensen, P. H., Nielsen, M. S., Jakes, R., Dotti, C. G., & Goedert, M. (1998). Binding of α-synuclein to brain vesicles is abolished by familial Parkinson's disease mutation. The Journal of Biological Chemistry, 273, 26292-26294

Jo, E., Fuller, N., Rand, R., George-Hyslop, P.S., & Fraser, P.E. (2002). Defective membrane interactions of familial Parkinson's disease mutant A30P α-synuclein. Journal of Molecular Biology, 315, 799-807.

Kahle, P. J., Neumann, M., Ozmen, L., Muller, V., Jacobsen, H., Schindzielorz, A., … Haass, C. (2000). Subcellular localization of wild-type and Parkinson's disease-associated mutant α-synuclein in human and transgenic mouse brain. The Journal of Neuroscience, 20, 6365-6373.

Kitada, T., Asakawa, S., Hattori, N., Matsumine, H., Yamamura, Y., Minoshima, S., Yokochi, M., Mizuno, Y., and Shimizu, N. (1998). Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. Nature, 392, 605-608.

Kim, H., Kim J., Lee, J., Park, S., & Jeon, B. (2010). Alpha-synulcein polymorphism and Parkinson's disease in a tau homogeneous population. Neurology Asia, 15, 61-63.

Kamiyoshihara, T., Kojima, M., Ueda, K., Tashiro, M., & Shimotakahara, S. (2007). Observation of multiple intermediates in α-synuclein fibril formation by singular value decomposition analysis. Biochemical and Biophysical Research Communication, 355, 398-403.

Kiely, A. P., Asi, Y. T., Kara, E., Limousin, P., Ling, H., Lewis, P., … Holton, J. L. (2013). α-Synucleinopathy associated with G51D SNCA mutation: a link between Parkinson's disease and multiple system atrophy? Acta Neuropathologica, Published online DOI: 10.1007/s00401-013-1096-7.

Kitada, T., Pisani, A., Porter, D. R., Yamasuchi, H., Tscherter, A., Martella, G., … Shen, J. (2007). Impaired dopamine release and synaptic plasticity in the striatum of PINK1-deficient mice. Proceedings of the National Academy of Sciences, 104, 11441-11446.

Kruger, R., Kuhn, W., Muller, T., Woitalla, D., Graeber, M., Kosel, S., … Riess, O. (1998). Ala30Pro mutation in the gene encoding alpha-synuclein in parkinson's disease. Nature Genetics, 18, 106-108.

Langston, J. W., Ballard, P., Tetrud, J. W., & Irwin, I. (1983). Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. Science, 219, 979-980.

Langston, J. W., Langston, E. B., & Irwin, I. (1984). MPTP-induced parkinsonism in human and non-human primates--clinical and experimental aspects. Acta Neurologica Scandinavica, Supplementum, 100, 49-54.

Lelan, F., Boyer, C., Thinard, R., Remy, S., Usal, C., Tesson, L, … Lescaudron, L. (2011). Effects of human alpha-synuclein A53T-A30P mutation on SVZ and local olfactory bulb cell proliferation in a transgenic rat model of Parkinson's disease. Parkinson's Disease, 1-11.

Li, W., West, N., Colla, E., Pletnikova, O., Troncoso, J. C., Marsh, L., & Lee, M. K. (2005). Aggregation promoting C-terminal truncation of α-synuclein is a normal cellular process and is enhanced by the familial Parkinson's disease-linked mutations. Proceedings of the National Academy of Sciences of the United States of America, 102, 2162-2167.

Liu, G., Qu, J., Suzuki, K., Nivet, E., Li, M., Montserrat, N., … Belmonte, J. C. I. (2012). Letter: Progressive degeneration of human neural stem cells caused by pathogenic LRRK2. Nature, 491, 603-607.

Liu, Y., Fallon, L., Lashuel, H.A., Liu, Z., & Lansbury Jr., P.T. (2002). The UCH-L1 gene encodes two opposing enzymatic activities that affect alpha-synuclein degradation and Parkinson's disease susceptibility. Cell, 111, 209-218.

Liu, C. W., Giasson, B. I., Lewis, K. A., Lee, V. M., Demartino, G. N., & Thomas, P. J. (2005). A precipitating role for truncated alpha-synuclein and the proteasome in alpha-synuclein aggregation: implications for pathogenesis of Parkinson disease. The Journal of Biological Chemistry, 280, 22670-8.

Lucking, C. B., & Brice, A. (2000). Review: Alpha-synuclein and Parkinson's disease. Cellular and Molecular Life Sciences, 57, 1894-1908.

Maguire-Zeiss, K. A., Short, D. W., & Federoff, H. J. (2005). Synuclein, dopamine and oxidative stress: co-conspirators in Parkinson's disease? Molecular Brain Research, 134, 18-23.

McLean, P. J., Kawamata, H., Ribich, S., & Hyman, B.T. (2000). Membrane association and protein conformation of α-synuclein in intact neurons. The Journal of Biological Chemistry, 275, 8812-8816.

McLean, J. R., Hallett, P. J., Cooper, O., Stanley, M., Isacson, O. (2012). Transcript expression levels of full-length alpha-synuclein and its three alternatively spliced variants in Parkinson's disease brain regions and in a transgenic mouse model of alpha-synuclein overexpression. Molecular and Cellular Neuroscience, 49, 230-239.

Myhre, R., Toft, M., Kachergus, J., Hulihan, M., Aasly, J. O., Klungland, H., & Farrer, M. J. (2008). Multiple alpha-synuclein gene polymorphisms are associated with Parkinson's disease in a Norwegian population. Acta Neurologica Scaninavica, 118, 320-327.

National Institute of Neurological Disorders and Stroke (2004). Parkinson's disease: challenges, progress and promise. Retrieved from URL http://www.ninds.nih.gov/disorders/parkinsons_disease/parkinsons_research.

Neuytemans, K., Theuns, J., Cruts, M., & Van Broeckhoven, C. (2010). Genetic Etiology of Parkinson Disease Associated with Mutations in the SNCA, PARK2, PINK1, PARK7, and LRRK2 Genes: A mutation Update. Human Mutation, 764-780.

Olanow, C. W., & Tatton, W. G. (1999). Etiology and pathogenesis of Parkinson's disease. Annual Review of Neuroscience, 22, 123-144.

Outeiro, T.F., Lindquist, S. (2003). Yeast Cells Provide Insight into Alpha- Synuclein Biology and Pathobiology. Science, 302(5651), 1772-1775.

Paisan-Ruiz, C., Jain, S., Evans, E. W., Gilks, W. P., Simon, J., van der Brug, M., … Singleton, A. B. (2004). Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. Neuron, 44, 595-600.

Paleologou, K. E., Oueslati, A., Shakked, G., Rospigliosi, C. C., Kim, H. Y., Lamberto, G. R., … Lashuel, H. A. (2010). Phosphorylation at S87 is enhanced in synucleinopathies, inhibits alpha-synuclein oligomerization, and influences synuclein-membrane interactions. The Journal of Neuroscience, 30 (9), 3184-3198.

Pandey, N., Schimidt, R. E., & Galvin, J.E. (2005). The alpha-synuclein mutation E64K promotes aggregation in cultured cells. Experimental Neurology, 1-6.

Parkinson's Disease Foundation. (2012). Statistics on Parkinson's, Retrieved from URL http://www.pdf.org/en/parkinson_statistics

Parkinson, J. (1817). An essay on the shaking palsy. London: Whittingham and Rowland.

Polymeropoulos, M. H, Lavedan, C., Leroy, E., Ide, S. E., Dehejia, A., Dultra, A., … Nussbaum, R. L. (1997). Mutation in the α-synuclein gene identified in families with Parkinson's disease. Science, 276, 2045-2047

Prasad, K., Tarasewicz, E., Strickland, P., O'Neil, M., Mitchell, S., & Mechant, K., (2011). Biochemical and morphological consequences of human a-synuclein expression in a mouse a-synuclein null background. European Journal of Science, 33, 642-656.

Purves, D., Augustine, G., Fitzpatrick, D., Hall, W., Lamantia, A., McNamara, J., & White, L. (2012). Neuroscience, Fifth Edition, Sinauer Associates.

Raaij, M. E. V., Segers-Nolten, I. M. J., & Subramaniam, V. (2006). Quantitative morphological analysis reveals ultrastructual diversity of amyloid fibrils from α-synuclein mutants. Biophysical Journal: Biophysical Letters, 96-98.

Ross, C. A., & Poirier, M. A. (2004). Protein aggregation and neurodegenerative disease, Nature Medicine, 10, S10-7.

Schwede, T., Kopp, J., Guex, N. & Peitsch, M. C. (2003). SWISS-MODEL: an automated protein homology-modeling server. Nucleic Acids Research, 31, 3381-3385.

Sian, J., Youdim, M., Riederer, P., & Gerlach, M. (1999). MPTP-Induced Parkinsonian Syndrome. Basic Neurochemistry: Molecular, Cellular and Medical Aspects. Philadelphia: Lippincott-Raven. Available from: http://www.ncbi.nlm.nih.gov/books/NBK27974/

Sharma, N., Brandis, K. A., Herrera, S. K., Johnson, B. E., Vaidya, T., Shrestha, R., & DebBurman, S. K. (2006). α-Synuclein budding yeast model toxicity enhanced by impaired proteasome and oxidative Stress. Journal of Molecular Neuroscience, 28, 161-178.

Sharon, R., Bar-Joseph, I., Frosch, M. P., Walsh, D. M, Hamilton, J. A., & Selkoe, D. J. (2003). The formation of highly soluble oligomers of α-synuclein is regulated by fatty acids and enhanced in Parkinson's disease. Neuron, 37, 583-595.

Sharon, R., Goldberg, M. S., Josef, I. B., Betensky, R. A., Shen, J., & Selkoe, D. J. (2001). α-synuclein occurs in lipid-rich high molecular weight complexes, binds fatty acids, and shows homology to the fatty acid- binding proteins. Proceedings of the National Academy of Sciences, 98, 9110-9115.

Shin, J., Dawson, V., & Dawson, T. (2009). SnapShot: pathogenesis of Parkinson's disease. Cell, 139, 440.

Spillantini, A. G., Crowther R. A., Jakes R., Hasegawa, M., & Goedert M. (1998). α-Synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with Lewy bodies. Proceedings of the National Academy of Sciences, 95, 6469-6473.

Taylor, J. P., Hardy, J., & Fischbeck, K. H. (2002). Toxic proteins in neurodegenerative disease. Science 296, 1991-1995.

Valente, E. M., Abou-Sleiman, P. M., Caputo, V., Muqit, M. M., Harvey, K., Gispert, S., … Wood, N. W. (2004). Hereditary early-onset Parkinson's disease caused by mutations in PINK1. Science, 304, 1158-1160

Vriend, G. (1990). WHAT IF: A molecular modeling and drug design program, Journal of Molecular Graphics, 8, 52-56.

Wersinger, C., Prou, D., Vernier, P., Niznik, H. B., & Sidhu, A. (2003). Mutations in the lipid-binding domain of α-Synuclein confer overlapping yet distinct, functional properties in the regulation of dopamine transporter activity. Molecular and Cellular Neuroscience, 24, 91-105.

Wilkinson, K., Knopacki, F., & Henley. J. (2012). Modification and movement: Phosphorylation and SUMOylation regulate endocytosis of GluK2-containing kainate receptors. Communicative &Integrative Biology, 5, 223-226

Xiong, L., Zhao, P., Guo, Z., Zhang, J., Li, D., & Mao, C. (2010). Alpha-synuclein gene structure, evolution and protein aggregation. Neural Regeneration Research, 5, 1423-1428.

Zarranz, J. J., Alegre, J., Gomez-Esteban, J. C., Lezcano, E., Ros, R., Ampuero, I., … de Yebenes, J. G. (2004). The new mutation, E46K, of alpha-synuclein causes Parkinson and Lewy body dementia. Annals of Neurology, 55, 164-173.

To view the full text, go to www.lakeforest.edu/eukaryon