α-Synuclein Misfolding and Aggregation in Parkinson's Disease

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Summary

Parkinson's disease (PD) is a fatal neurodegenerative disorder that affects 1.5 millions Americans and 1 in 100 individuals over the age of 60. It results from neuronal atrophy localized within the substantia nigra *pars compacta*. Upon autopsy, PD patients have large intraneuronal fibrils, Lewy Bodies, composed of α-synuclein. Familial forms of PD result from the A30P and A53T mutations within α-synuclein. Wild-type (WT), A30P, and A53T-α-synuclein aggregate from monomers into protofibrils before forming fibrils. Previously, fibrils were thought to be the PD causative agent however, recent evidence suggests that the protofibril may be the true toxic conformation of α-synuclein. In 2004, Zarranz et al. discovered a novel mutation, α-synuclein-E46K, in a Spanish family. Little is known about this mutation, except that it increases the rate and quantity of fibril formation as well as the lipid binding affinity of α-synuclein. The fibril formation pathway for E46K is unknown, though it likely includes a protofibrillar intermediate. Future research is needed to characterize E46K and compare it to the other familial mutants. Treatment approaches aimed at reducing the concentration of protofibrils could be accomplished through accelerated fibril formation, decreased α-synuclein expression, increased α-synuclein degradation, or reduction of intracellular dopamine which binds and stabilizes protofibril.

Introduction

Parkinson's disease (PD) is a fatal neurodegenerative disorder of the central nervous system. It is characterized by motor initiation deficits, rigidity, bradykinesia, and resting tremor. It affects 1 in 100 individuals over the age of 60 and 90 to 95% of all PD cases are sporadic (NPD Foundation, 2006). Heritable forms of the disease constitute the remaining 5-10% of cases (NPD Foundation, 2006). Within the whole of PD cases, another 5 to 10% occur in individuals under the age of 40 (NPD Foundation 2006). For the past decade, millions of dollars in federal and private funding along with numerous researchers have provided us with a large body of knowledge on PD. However, there is still no cure and all patients are destined to die as a result.

Pathology

As Dr. James Parkinson first observed in 1817, the PD brain is characterized by neuronal atrophy localized within the *pars compacta* region of the substantia nigra. Most PD patients do not display any symptoms until 60-80% of substantia nigral neurons are dead (Purves et al., 2004). These neurons have a black coloration because they contain the molecule melanin (Purves et al., 2004). Upon autopsy, little pigmentation is observed in the substantia nigra, which

indicates degeneration. At the cellular level, substantianigral neurons contain large intraneuronal aggregates, Lewy Bodies, which consist primarily of the protein α-synuclein (Spillantini et al., 1998). Interestingly, aggregates are found only in these neurons even though α-synuclein is abundantly expressed throughout the human brain.

In comparison of PD with the neurodegenerative disorders Alzheimer's disease (AD), and Amyotrophic Lateral Sclerosis (ALS, Lou Gehrig's), a common pathology is observed (Lansbury et al., 2003). Upon autopsy, the AD brain is characterized by neuronal atrophy localized within the medial temporal lobe. Like PD patients, the medial temporal neurons in AD contain large intraneuronal aggregates composed of the protein tau, as well as extra-cellular β-amyloid plaques (Lansbury et al., 2003). ALS patients have degeneration of α-motor neurons which also exhibit large intraneuronal aggregates consisting of superoxide dismutase-1 (SOD1) (Ray et al., 2004). Overall, each of these diseases has a common pathology of protein misfolding and aggregation.

This review will focus on the characteristics of α-synuclein, primarily misfolding and aggregation. However, many of the characteristics of α-synuclein provide valuable insight into the proteins involved in AD and ALS. Significant commonalities will be referred to within the text.

Biological Basis

The actual biological basis of PD remains to be discovered, however, mutations within several proteins have been linked to familial forms of PD. PD results from the mutations A30P (Krueger et al., 1998), A53T (Polymeropoulos et al., 1997), or E46K (Zarranz et al., 2004) within the protein α-synuclein. It can also result from mutations within *parkin* (Kitada et al., 1998), UCH-L1 (Leroy et al., 1998), PINK1 (Valente et al., 2004), DJ-1 (Bonifati et al., 2003), and LRRK2 (Funayama et al., 2002). Because A30P, A53T, and E46K mutations cause PD and α-synuclein is the primary component of Lewy Bodies, it has remained the main target of research.

Systems for Modeling PD

Several different systems are used for the study of PD including mice, drosophila, yeast, primary neuron cultures, and various *in vitro* techniques. A line of transgenic mice overexpressing human α-synuclein has been established, and found to exhibit substantia nigral atrophy and motor deficits similar to those in PD (Masliah et al., 2000). Interestingly, these mice have Lewy Body like structures that differ from those found in human neurons because they are non-fibrillar (unorganized) aggregates (Goldberg and Lansbury, 2000). In contrast, the α-synuclein aggregates in humans are highly organized fibrils (Lansbury et al., 2003). Mice expressing both human α-synuclein and its homologue, β-synuclein, have fewer aggregates and show no symptoms of PD (Hashimoto et al., 2001). A drosophila model expressing human wild-type (WT), A30P, and A53T α-synuclein has also been synthesized and found to exhibit aggregation, dopaminergic neuronal atrophy, and motor deficits (Feany and Bender, 2000). Finally, several yeast models expressing human α-synuclein-WT, A30P, and A53T have been established (Outeiro et al., 2003; Dixon et al., 2005; Zabrocki et al., 2005; Sharma et al., 2006; Brandis et al., 2006).

α-Synuclein

As previously mentioned, α-synuclein is the principal component of Lewy Bodies and causes PD when A30P, A53T, or E46K mutations are present. It is a small, 140 amino acid, 14 kDa, natively unfolded protein, but highly α-helical in the presence of fluorinated alcohols, detergents, or vesicles (Weinreb et al., 1996; Davidson et al., 1998). α-synuclein is divided into three distinctive regions. The N-terminus is amphipathic and consists of amino acids 1-60, which contain seven highly conserved 11-amino acid slightly variable repeats, XKTKEGVXXXX (Kessler et al., 2003; Uversky et al., 2002). The central portion of α-synuclein is amyloidagenic and consists of amino acids 61-95. It contains two of the above repeats above (Uversky et al., 2002). Finally, the C-terminus consists of amino acids 96-140 and contains many acidic residues as well as numerous prolines which are characteristic of unfolded proteins (Uversky et al., 2002). α-synuclein is expressed throughout human brains primarily in pre-synaptic terminals. There it may be involved in vesicular trafficking (Maroteaux et al., 1988), regulation of neurotransmitter (Jensen et al., 1998; Murphy et al., 2000) as well as several other possible functions not listed. The actual function of α-synuclein remains unknown.

A30P, A53T and Fibrillization

Lewy Bodies are the end products of monomeric α-synuclein aggregation and are present in every PD brain (Purves et al., 2004). Initial studies of α-synuclein were directed at determining the mechanism by which it transformed from monomers into highly organized fibrils. In order to begin this enormous task, Conway et al. conducted an *in vitro* analysis of A30P, and A53T to determine their rates of aggregation (1998). At low concentrations of monomer, they found both isomers to be natively unfolded. However, as their concentrations increased fibrils began to form at different rates depending on the isoform, with A30P fibrillizing the slowest and A53T the fastest (Conway et al., 1998). In addition to increases in monomer concentration, increased molecular crowding increases the rate of fibrillization (Shtilerman et al., 2002). The Conway et al. study led to the hypothesis that familial PD resulted from increased aggregation of α-synuclein (1998).

Discovery and Properties of Protofibrils

This "old" model quickly changed when Conway et al. conducted a subsequent experiment that specifically quantified the rate of monomeric α-synuclein disappearance and rate of fibril formation (2000). At equimolar concentrations of A30P, A53T, WT, the rates of monomeric A30P and A53T consumption were increased compared to WT with A53T being the fastest. In addition, a mixture of A30P and WT was consumed slower than A30P alone and A53T mixed with WT. Interestingly, the rates of fibril formation for both A53T and A30P did not account for their rates of monomeric disappearance. Thus, Conway et al. hypothesized that a protofibrillar intermediate existed (2000). The existence of the protofibril was confirmed using atomic force microscopy (AFM) and gel filtration chromatography on solutions of A30P, and A53T (Conway et al., 2000). Gel filtration isolated oligomeric intermediates between monomer and fibril forms. AFM of these protofibrils revealed them to exist in either a spherical conformation, chain of spheres, or rings of spheres. Thus, Conway et al. revised their previous hypothesis by proposing that a protofibrillar intermediate was the causative agent in PD (2000). If protofibrils were the

toxic form of α-synuclein, it would account for the lack of A30P propensity for aggregation yet still being capable of causing PD.

Following the discovery of protofibrils, a study conducted by Rochet et al. compared the in vitro fibrillization properties of human α-synuclein-WT, A30P, and A53T to mouse α-synuclein (2000). Like human α-synuclein, mouse α-synuclein exists in a natively unfolded structure, and has a threonine amino acid at the 53rd codon (Rochet et al., 2000). Mouse α-synuclein aggregates even faster than human A53T (Rochet et al., 2000). However, mouse aggregates are similar because they are β-sheet rich (Rochet et al., 2000). Interestingly, mixtures of mouse α-synuclein with human WT or A53T led to the slowing of fibrillization but an increase in protofibril formation (Rochet et al., 2000). These findings further support the hypothesis of a toxic protofibrillar intermediate because transgenic mice can express PD symptoms without organized fibril formation (Goldberg and Lansbury, 2000).

α-Synuclein Membrane Affinity

In addition to the folding, misfolding, and aggregative properties of α-synuclein, it also closely associates with lipids (Davidson et al., 1998; Jensen et al., 1998; Bussell, et al., 2004; Sharma et al., 2006). Lipid affinity may be the result of α-synuclein's numerous 11-amino acid repeats which are characteristic of many apolipoproteins (Davidson et al., 1998).

Detailed analysis of α-synuclein phospholipid interaction by Davidson et al. demonstrated that the α-synuclein secondary structure stabilized into an α-helical conformation following binding to phospholipid bilayers (1998). Furthermore, this experiment was conducted on a variety of vesicular sizes which revealed α-synuclein to preferentially bind to those 20-25 nm in diameter. Similar studies using the familial mutants A30P and A53T found α-synuclein membrane affinity to differ between the familial mutants (Jensen et al., 1998, Perrin et al., 2000). The A30P mutation decreases α-synuclein's ability to bind to membranes whereas A53T affinity is similar to WT (Jensen et al., 1998, Perrin et al., 2000). These observations are consistent with those found in a budding yeast model (Sharma et al., 2006). In budding yeast, WT and A53T are both found to membrane localize whereas A30P is observed to remain diffuse (Sharma et al., 2006).

In depth analyses of the α-synuclein secondary structure have revealed the N-terminus to bind membranes while the C-terminus remains unbound and unfolded (Eliezer et al., 2001). Specifically, amino acids 1-102 confer α-synuclein membrane binding capabilities (Perrin et al., 2000). Because both A30P and A53T reside within this domain, it is not surprising that A30P diminishes membrane binding capability. High resolution NMR spectroscopy of α-synuclein confirmed that the N-terminus binds to lipid membranes and consequently acquires a helical conformation (Eliezer et al., 2001). Interestingly, the C-terminus remains unbound and unfolded suggesting it may be involved in binding to other molecules or vesicles (Eliezer et al., 2001).

Thus, it is plausible that α-synuclein is involved in vesicular traffic at the pre-synaptic terminal based on two lines of evidence; 1) α-synuclein preferentially binds to vesicles 20-25 nm in diameter, which corresponds to the size of those carrying neurotransmitter (Davidson et al., 1998) and 2) the C-terminus remains unfolded and free to associate with other vesicles or molecules (Eliezer et al., 2001).
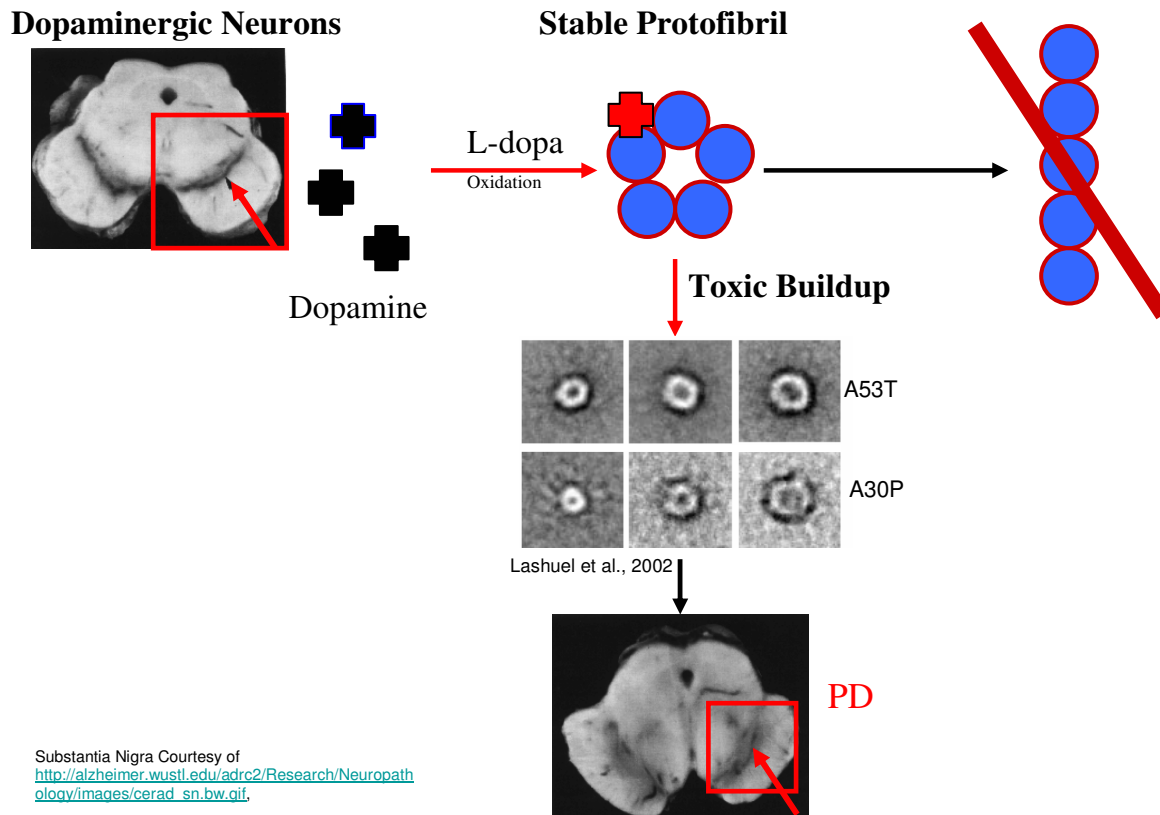
Figure 1: This figure illustrates the possible relationship between PD and the substantia nigra. L-dopa is an oxidized form of dopamine that has been binds and stabilizes protofibrils (Lashuel et al., 2002). In this model, L-dopa (red) binds and stabilizes protofibrils. The stabilized protofibrils have a high affinity for membranes and form pores similar to those of bacterial toxins which are depicted as AFM images from Lashuel et al., 2002. Those pores may alter the permeability of substantial nigral neurons consequently, causing PD.

α-Synuclein Post-Translational Modification

In a transgenic mouse model for PD, it was shown that α-synuclein is post-translationally modified into an N-terminally truncated variant found only in regions of neurodegeneration (Lee et al., 2002; Kessler et al., 2003). Based on this observation and that the N-terminus confers several of α-synuclein's characteristics (previously described), Kessler et al. evaluated the significance of seven N-terminus repeat sequences (2003). Two α-synuclein variations were synthesized; one with the addition of two additional repeats, and the other with two fewer repeats (both in the N-terminus) (Kessler et al., 2003). Interestingly, their results indicated that the plus two mutants prefer a non-amyloid, α-helical conformation while the minus two acquired a β-sheet rich conformation. This finding is significant because potentially pathogenic protofibrils and fibrils are β-sheet rich (Lansbury et al., 2003). Therefore, the repeat sequences may have been highly conserved in order to protect against aggregation (Kessler et al., 2003).

In AD, the APP is post-translationally modified into two fragments, Aβ40 and Aβ42, which are linked to familial forms of the disease (Lansbury et al., 2003). It is feasible that a mechanism similar to this one may occur in PD patients, producing toxic truncated variations of α-synuclein (Kessler et al., 2003).

Protofibril Membrane Affinity

The pathway toward Lewy Body formation has been elucidated for WT, A30P, and A53T; however, the pathogenic agent has not. Of all three (monomer, protofibril, and fibril) conformations α-synuclein is known to exist in, emerging evidence has indicated that the protofibril is the toxic agent. Critical to this hypothesis was an experiment by Volles et al. using circular dichromism (2001). They found α-synuclein protofibrils to bind in a β-sheet rich structure to membranes isolated from rat brains. Significantly, AFM revealed protofibrils to bind to membranes and permeabilize them (Volles et al., 2001). Another study conducted by Ding et al. compared the binding affinity of protofibril and monomeric α-synuclein to synthetic vesicles, rat-brain derived membranes, and mitochondria (2002). Though both monomeric and protofibril α-synuclein bind membranes, protofibils bound to each membrane more tightly than the monomers (Ding et al., 2002). Interestingly, protofibrils formed pores resembling those of bacterial toxins (Volles et al., 2001). In addition, both A30P and A53T permeabilization was greater per mole of protofibrils compared to WT (Volles et al., 2001). Unlike protofibrils and monomers, fibrils do not bind membranes (Volles et al., 2001; Ding et al., 2002). Together, these studies provide evidence supporting the toxic protofibril hypothesis (Figure 1).

Recently Discovered E46K

In 2004, Zarranz et al. discovered the autosomal dominant familial PD mutation α-synuclein-E46K in a Spanish family (2004). Since its discovery, there have only been five published manuscripts on E46K out of the thousands of PD papers. The first in vitro study examined the rate of E46K

fibrillization, and lipid binding affinity (Choi et al., 2004). E46K formed aggregates at a faster rate than WT but similar to A53T (Choi et al., 2004). The E46K fibrils were also conformationally different than A30P and A53T (Choi et al., 2004). In addition, E46K increased α-synuclein vesicular binding affinity whereas A30P decreased it significantly, and A53T slightly (Choi et al., 2004).

Greenbaum et al. demonstrated that E46K increases α-synuclein's rate of fibrillization as well (2005). Time-course circular dichromism analysis of the E46K fibrillization process revealed α-synuclein to transition from primarily α-helical to β-sheet as fibrils formed (Greenbaum et al., 2005). This transition occurred more rapidly for E46K compared to WT (Greenbaum et al., 2005).

A single in vivo analysis of E46K aggregation was performed by Pandey et al. using human catecholaminergic neuroblastoma cells (2006). They demonstrated that 18% of E46K, 12% of A53T, 2% of A30P, and 6% of WT expressing cells contained aggregates. Electron microscopy identified two types of aggregates within these neurons (Pandey et al., 2006). Furthermore, western analysis showed that E46K formed greater quantities of aggregates compared to A53T thus, indicating the higher propensity for E46K to aggregate (Pandey et al., 2006).

The β-sheet nature of E46K aggregates is similar to those of A30P and A53T (Conway et al., 2000; Greenbaum et al., 2005). Therefore, it is probable that E46K forms β-sheet rich protofibrils before fibrils as well (Figure 2). However, detailed analysis of E46K fibrillization still remains to be conducted.

α and β Synucleins

The synuclein family consists of α, β, and γ homologues, all of which are expressed in humans (Park et al., 2003). α and β synucleins are primarily expressed in the brain whereas γ-synuclein is in the peripheral nervous system (Park et al.,

2003). A relationship has been established between α and β-synuclein co-expression and α-synuclein aggregation (Hashimoto et al., 2001; Park et al., 2003). Double-transgenic mice expressing both human α and β-synucleins do not exhibit neuronal atrophy, aggregates, or motor deficits (Hashimoto et al., 2001). In vitro, β-synuclein prevents α-synuclein-A53T from forming protofibrils and fibrils (Park et al., 2003). Analysis of β-synuclein's relationship with the other familial mutants has yet to be performed.

PD Specificity for the Substantia Nigra

Though α-synuclein is abundantly expressed in the human brain, neurodegeneration is localized to the substantia nigra (Purves et al., 2004). Conway et al. performed a wide screen of pharmaceuticals to find molecules that prevent fibril formation (2001). Interestingly, catecholamines related to dopamine act as inhibitors of fibrillization (Conway et al., 2001). Furthermore, an oxidized form of dopamine, L-dopa, bound and stabilized protofibrils (Conway et al., 2001). Under oxidative conditions within substantia nigral neurons it is feasible that toxic protofibrils accumulate and are stabilized by L-dopa, consequently linking neurodegeneration to the substantia nigra (Conway et al., 2001)

Possibilities for Treatment

After reviewing the characteristics of α-synuclein and its relationship to PD, several possibilities for treatment arise. Currently, PD is widely treated by replenishing the substantia nigra synaptic clefts with dopamine (Purves et al., 2004). However, this neither cures nor prevents neurodegeneration. In order to prevent or inhibit neurodegeneration, the concentration of dopamine in the cytoplasm could be reduced (Conway et al., 2001). This would decrease the concentration of potentially toxic protofibrils and
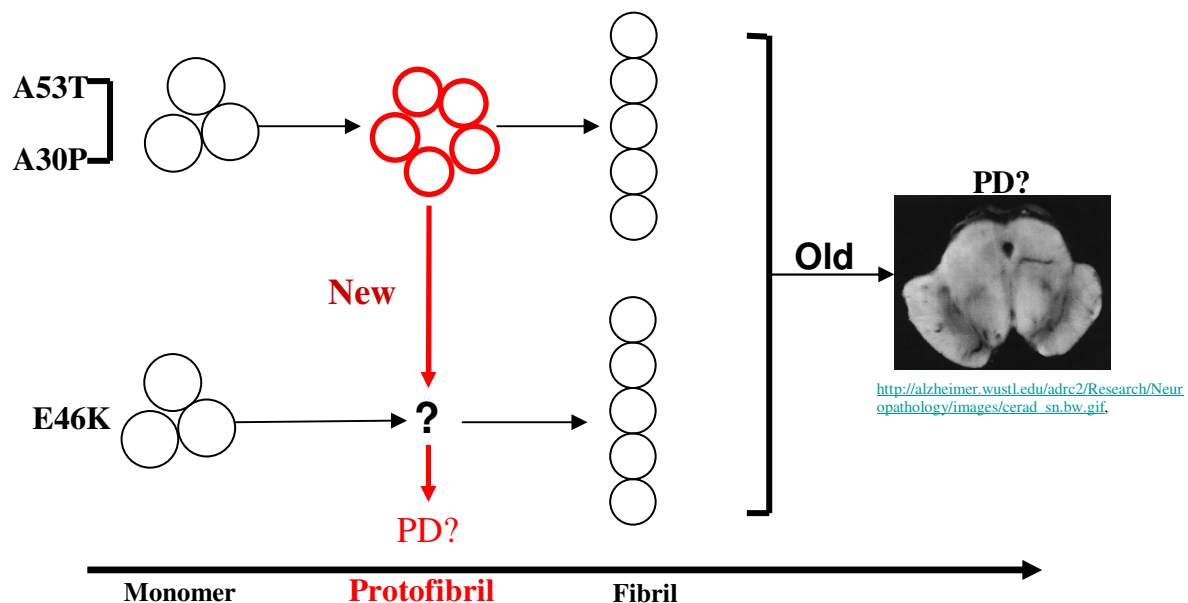

Figure 2: Comparison of the toxic protofibrillar hypothesis ("New") to the toxic fibril hypothesis ("Old"). The upper portion of the scheme depicts the two previously discovered familial mutants A30P and A53T, and their pathway toward fibril formation ending in possible neurodegeneration. The lower part of the diagram shows E46K and what is known of it's aggregation pathway from monomer to fibril and possibly neurodegeneration. The E46K protofibril has yet to be discovered. The image on the right is of the substantia nigra from a PD brain upon autopsy. Adapted from White, 2006.

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consequently prevent neuronal atrophy (Conway et al., 2001). Because protofibrils are not necessarily the PD causative agent, therapeutics aimed at increasing the degradation of α-synuclein or reducing its expression may prevent buildup of the toxic species as well.

Conclusion

PD affects millions of individuals world wide and is always fatal. Familial forms of PD are known to result from the mutations A30P, A53T, and recently discovered E46K in α-synuclein (Krueger et al., 1998; Polymeropoulos et al., 1997; Zarranz et al., 2004). The transition from monomeric WT, A30P, and A53T to fibrillar α-synuclein includes a protofibrillar intermediate (Conway et al., 2000 and 2001). However, the pathway to fibril formation has not been determined for E46K. If E46K were to fibrillize without forming protofibrils, this would refute the toxic protofibril hypothesis. This possibility is unlikely because WT, A30P, and A53T all form protofibrils. Future research is needed to determine the characteristics of E46K and how they compare to those of A30P and A53T. Finally, the study of misfolding and aggregation of α-synuclein provides many valuable insights into PD as well as numerous other neurodegenerative disorders. Currently, protofibrils have been identified in AD and ALS patients as well (Harper et al., 1997; Lansbury et al. 2000; Ray et al., 2004).

Acknowledgements

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