

Development of a Model System to Analyze Factors in Parkinson's Disease - Like Protein Aggregation

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Aggregation of the protein α -synuclein is thought to be a main contributor in cell death of dopaminergic neurons, which leads to Parkinson's Disease (PD). Previous studies have shown that TorsinA, a protein associated with dystonia, co-localize with these aggregates in the human brain. The worm homologue of TorsinA, *tor-2*, has also previously been shown to reduce protein aggregates resembling those found in Huntington's disease, another neurodegenerative disease. We have created a model system of Parkinson's like protein aggregation in the nematode *C. elegans* in order to assess *tor-2*'s ability to also prevent or reduce α -synuclein aggregates. The model system will also be used to screen other genes and compounds capable of decreasing or increasing aggregation in hopes to identify possible therapeutics, understand the cellular mechanism of aggregation and recognize new compounds that might serve as environmental factors in the development of PD.

Introduction

Parkinson's Disease (PD) affects over 1,000,000 people in the United States alone. The disease is characterized by resting tremor, muscle rigidity and loss of muscular control. These symptoms are caused by a lack of dopamine that results from the specific death of dopaminergic nigrostriatal neurons in the substantia nigra pars compacta and cerebral cortex. The exact mechanism of cell death is still unknown but appears to be caused by the presence of Lewy bodies. These protein aggregates are composed primarily of α -synuclein (α -syn) but are also immunoreactive for ubiquitin, 3-nitrotyrosine, cyclin-dependent kinase 5, and TorsionA^{1,2}. α -syn is one of three proteins in the synuclein family. A portion of α -syn, the non-amalyoid precursor component, is also found in the α -amalyoid plaques, which are a pathology of Alzheimer's disease. Prior work done in the Caldwell lab has shown that *tor-2*, the worm homologue of the human protein TorsinA, which causes the movement disorder dystonia when defective, is capable of alleviating protein aggregation that mimics that of Huntington's disease, another neurological degenerative protein

aggregation disease³. *tor-2* is part of the HSP family that are thought to be chaperones, proteins that functions to properly fold other proteins and refold misshapen proteins¹. We are particularly hopeful of its ability to act on Lewy Body like aggregates since TorsinA, the human analog was recently found to accumulate in Lewy Bodies found in cases of sporadic Parkinson's disease.¹

A few mutations in the α -syn protein have been linked to familial cases of PD. We have used wild type α -syn as well as three such mutations, [A53T], [A30P], and [Δ 73-83] in our fusion to GFP. Each of these mutations is dominant and results in early onset PD. However, these mutations account for only ~1% of all cases. The vast majority of cases are the products of either other gene defects, environmental factors or other unknowns.

Epidemiological studies have implicated both pesticides and heavy metals in the development of PD. Previous studies have already assessed the affects of rotenone⁵ and paraquat as well as many heavy metal ions including Fe³⁺, Cu²⁺, Zn²⁺⁶. This model of PD protein aggregates will allow quick and easy screening of other

compounds that can either increase or decrease the protein aggregation.

Materials and Methods

Wild-type *C. elegans* strain N2 (Bristol Variety) was cultured at 20°C under standard feeding conditions⁶. α -syn supplied by Philipp Kahle of the Laboratory for Alzheimer's and Parkinson's Disease research at the Ludwig Maximilians Universitat Munchen.

Cloning: pfx Taq used in all PCRs. Primers with an Xba cut site for the 5' portion of α -syn 5'-GCTCTAGAATGGA CGTGTTTCATGAAGGGC and a Bam site for the 3' end 5'-CGGGATCCGCCTCT GGCTCATACTCCTG were used to clone all four α -syn cDNAs and fused to GFP in pPD95.75. Quiagen PCR cleanup and miniprep kits used with company protocol. Q-BIOgene's GeneCleanII® Kit and company protocol used for isolation and purification of gene fragments. Routine electroporation techniques used for transformation of *E. coli* at various stages in cloning process. Primers 5'-GGGGACAAGTTTGTACAAAAAAGCA GGCTACATGGACGTGTTTCATGAAGGGC and 5' - G G G G A C C A C T T T G T A C AAGAAAGCTGGGTCCTATTTGTATA GTTCATCCATGCC (Operon) used to place α -syn::GFP fusion under control of *unc-54* in pD30.38 using Invitrogen's Gateway system under guidance of Chris Gelwix. *tor-2* under control of *unc-54* was previously prepared by Elaina Sexton. GFP alone was also placed under control of *unc-54* using Gateway system and primers 5'-GGGGACAAGTTTGTACAAAAAAG CAGGCTCCATGAGTAAAGGAGAAGAAC and 5' - G G G G A C C A C T T T G T A C A GAAAGCTGGGTCTCATCCATGCCAT GTGTAATCCC.

Injection: Injection of concentrated α -syn::GFP under *unc-54* control with *rol-6* (gene marker) and currently the α -syn::GFP with *rol-6* and *tor-2*, into gonad of L4 worms performed with standard protocol by David Cao.

Production of Stable Lines: Worms were maintained for up to 7 generations to ensure some level of transmission of both the *rol-6* marker and fluorescence.

Irradiation: Permanent transgenic lines were produced by α -irradiating stable lines for 50 mins. with 3500-4000 rads from a Cobalt 60 source.

Examination: Fluorescence microscopy was performed on a Nikon Eclipse E800 epifluorescence microscope equipped with a Endow GFP HYQ cube (Chroma, Inc.) Worms were monitored at L1-Adult to see if protein aggregation increased with age.

Results and Discussion:

Wild type α -syn and the three previously mentioned mutations have been fused to GFP and placed under the control of the body wall muscle *unc-54*. All four constructs have been injected and irradiated to produce transgenic lines of the [A53T] and [A30P] constructs with the wild type and [α 73-83] constructs still in the screening process. Examination of animals at 400-1000X before the lines were irradiated indicated many aggregates within the body wall muscle (images 1-4). Injections are also under way of the four constructs with *tor-2* with a stable line so far produced for wild type α -syn & *tor-2*.

We are just entering the analysis stage of but so far the four constructs have produced aggregation in the body wall muscle the wild type α -syn & *tor-2* rescue appears to be completely void of aggregates (image 5/cover). A brief aging study using the two transgenic mutations seem to indicate that the aggregates worsen with age and would therefore mimic the human condition as well. Even though we are only in the beginning stages of evaluation, the system appears to be a successful model of Parkinson's like aggregations.

As we await the completion of the transgenic lines containing *tor-2* we are also beginning to look at compounds that have already been shown to increase aggregation of α -syn to see if our system reacts the same way. We are also searching for novel compounds that could also increase or even decrease the α -syn aggregations.

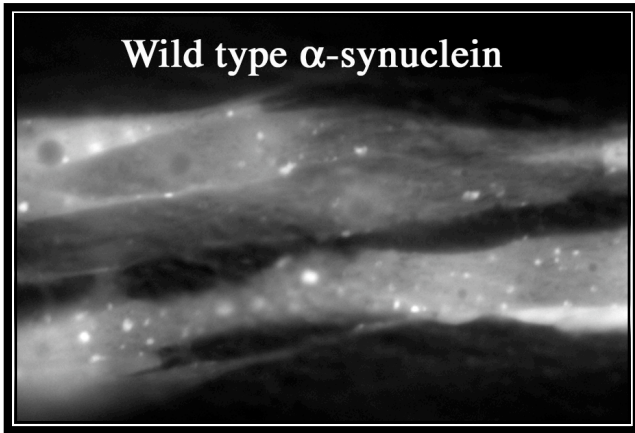


Image 1. Body wall muscle showing aggregates of w.t. α -syn::GFP under control of *unc-54* at 1000X.

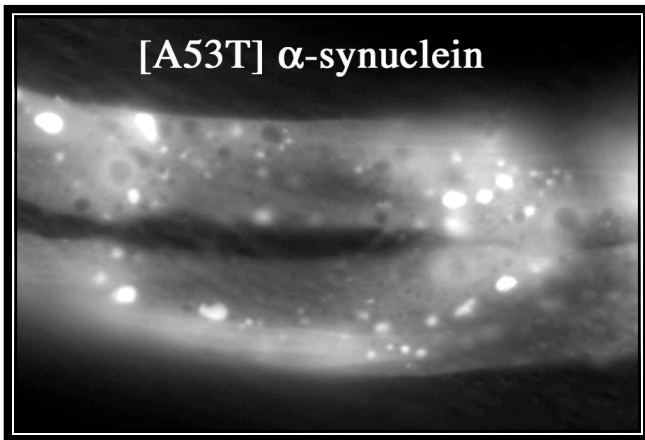


Image 2. Body wall muscle showing aggregates of the mutant [A53T] α -syn::GFP under control of *unc-54* at 1000X.

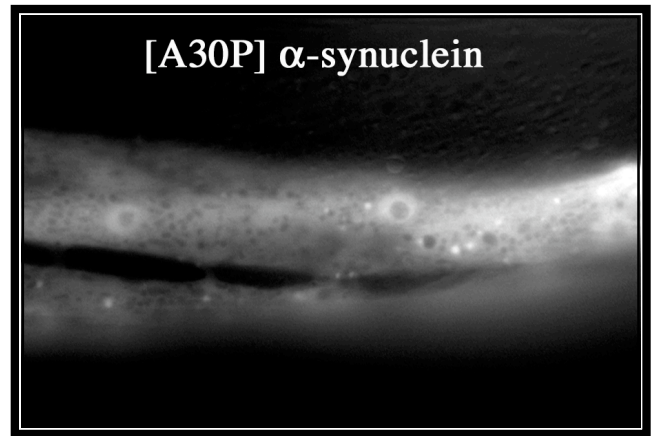


Image 3. Body wall muscle showing aggregates of the mutant [A30P] α -syn::GFP under control of *unc-54* at 1000X.

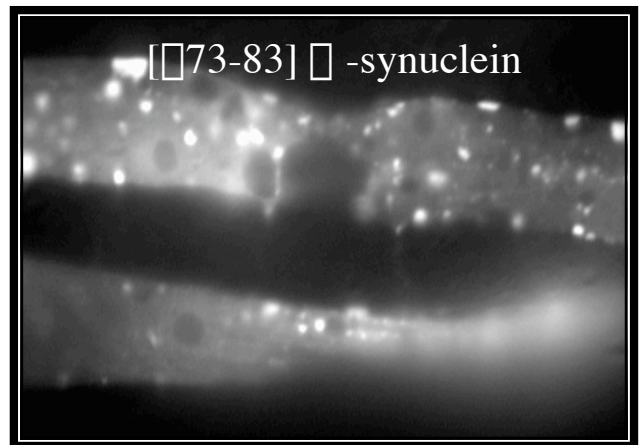


Image 4. Body wall muscle showing aggregates of the mutant [73-83] α -syn::GFP under control of *unc-54* at 1000X.

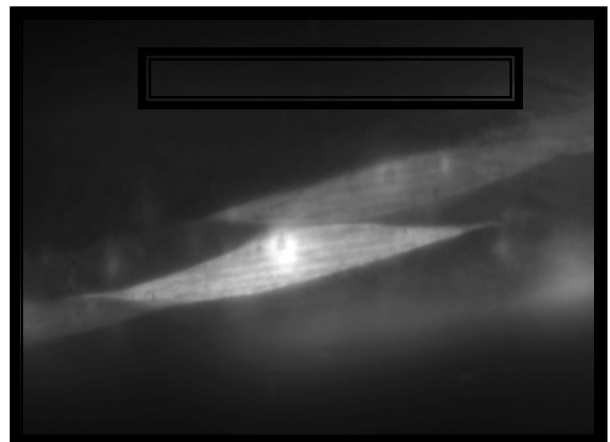


Image 5. Body wall muscle showing no aggregates, only fluorescence of the body wall muscle itself with the rescue of *tor-2* of w.t. α -syn::GFP at 1000x.

References

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