ABSTRACT

Parkinson’s disease (PD) is a hypokinetically neurodegenerative disorder characterised by the death of midbrain dopaminergic neurons. This selective cell death is linked to the misfolding and aggregation of the brain protein α-synuclein that accumulates in Lewy bodies. The full-length α-synuclein (140 amino acids long) associates with membranes and its C-terminus keeps it soluble. In PD, this full-length α-synuclein is the major component of Lewy bodies; however, several α-synuclein-truncation variants (e.g. A53T, 110, 123) were also recently found in the brain (Lee et al., 2010). While these variants can increase the aggregation of full-length α-synuclein in vitro and enhance toxicity in specialised cell lines (Li et al., 2005; Liu et al., 2005), the individual properties of each variant towards aggregation, membrane association, and toxicity in free living organisms is not well studied. In this three-year Richter Scholar project, we sought to test the hypothesis that the larger the truncations, the more the variants would reduce α-synuclein solubility and membranes association, and increase toxicity in organisms. Our goal was to create these four variants of α-synuclein (in both wild-type and two familial PD mutant versions: A30P and A53T) and characterise their properties in a budding yeast (Saccharomyces cerevisiae) model for PD. In this poster, we report the creation of all twelve variants and their successful transformation into yeast. The next goal is to evaluate several properties of these variants by comparing them to the full-length form: their cellular localization (GFP imaging), expression level (Western blotting), and toxicity (serial dilution growth on plates).

INTRODUCTION

Substantia nigra

Lewy body accumulation

Known. Truncated A53T has been the only familial mutant evaluated.

Gap:

Increase aggregation of full-length α-synuclein and oxidant accumulation, mitochondrial dysfunction, calcium Dysfunctional Pathways

HYPOTHESIS & AIMS

Hypothesis:
The larger the truncation, the more likely it enhances pathogenicity by increasing aggregation and toxicity; truncated familial mutants are even more pathogenic than the wild-type form.

Model organism: Budding yeast (S. cerevisiae)

Aim:
To create truncation variants of α-synuclein (αsyn 110, 123, 120, and 113) in wild-type and two familial mutants (A30P & A53T).

STRATEGY AND RESULTS

STEP 1

PCR: Primer & Template Preparation

STEP 2

PCR: Synthesis of Truncation Variant

STEP 3

Variant DNA Purification

STEP 4

Subcloning & Bacterial Transformation

STEP 5

Gene Orientation Verification

STEP 6

Truncation Variant Plasmid Preparation

STEP 7

Sequencing

STEP 8

Yeast Transformation

CONCLUSION

✓ We successfully created all twelve α-synuclein truncation variants by PCR and put them into yeast pYES2 vectors.

DISCUSSION

This three-week project was designed as a prototype digital research experience for first-year science majors at Lake Forest College chosen as Richter Scholars. This scholarship program selects the top 20 students who complete an original research project under the mentorship of a faculty member on an original research project during the summer.

For the past two years, the Richter program has been ten weeks long. This year, the college additionally piloted a three-week program to expand opportunities to top scholars and faculty. The project was designed as a prototype for original research experience for first-year students to pursue an original hypothesis-driven scientific research project within such a limited time and make significant contributions to the discovery process.

It's possible for first-year students to pursue an original hypothesis-driven scientific research project within such a limited time and make significant contributions to the discovery process.

FUTURE

These tools can be used for multiple avenues of research in yeast:

Properties
- Localization
- Aggregation
- Accumulation
- Toxicity

Dysfunctional Pathways
- Degradation
- Protein folding
- Oxidative stress

REFERENCES


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