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PMSF and SFN Reduce Alpha-synuclein Aggregation in a Yeast Model of Parkinson's Disease

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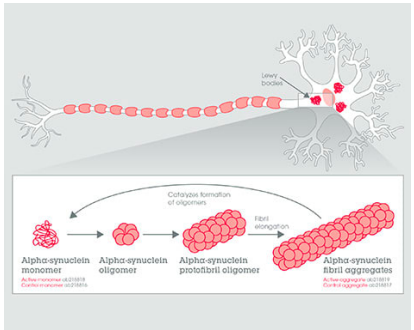
ABSTRACT

Parkinson's Disease, PD, is the second most common neurodegenerative disease in humans. PD is marked by Lewy body formation in the brain, which disturbs the dopamine transfer system across neurons. Previous studies have shown that the protein, α-Synuclein, is a major contributor in the formation of Lewy bodies. In this study, we modeled α-Synuclein aggregation in the Budding Yeast, Saccharomyces cerevisiae and treated the cells with Phenylmethylsulfonyl fluoride (PMSF) in one trial, and Sulforaphane (SFN) in another. Our goal was to see how PMSF and SFN might affect aggregation, while also monitoring the health of the yeast. Our Preliminary data has suggested that a 4mM concentration of PMSF and 200µg/ml of SFN significantly reduces protein aggregation. Our lab will continue to investigate the role of PMSF and SFN in the prevention and breakdown of α-Synuclein aggregates.

INTRODUCTION

The protein α-Synuclein is found in the neurons of mammalian cells. Although not fully understood, it has been hypothesized that α-Synuclein plays a role in membrane binding and the monitoring of neurotransmitter concentrations. Using yeast as a model organism has shown to be an affective way of observing these human proteins because of its ability to mimic the aggregation and cytotoxicity of α-Synuclein in human pathology. After imaging cells that were overexpressing α-Synuclein, drug therapies were introduced to see how they might affect protein aggregation. Phenylmethylsulfonyl fluoride (PMSF) and Sulforaphane (SFN) have shown the ability to alleviate protein aggregation.

FIGURE 1: Parkinson's Disease has been linked to alpha-synuclein aggregation in Lewy Bodies



[www.abcom.com]

FIGURE 2: Overexpression of Human α-synuclein in Yeast Triggers Aggregation

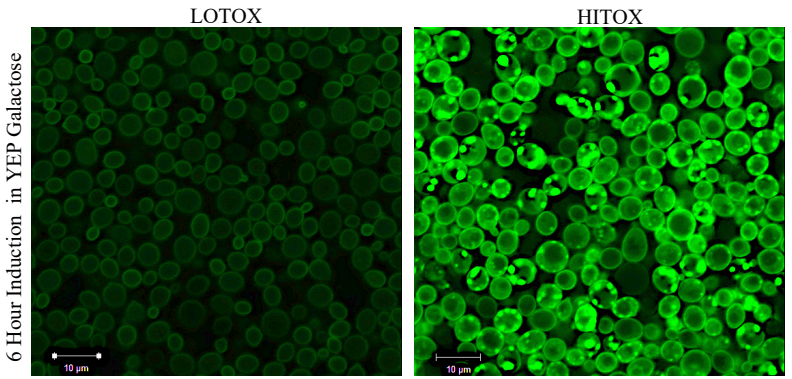


Figure 2: Two strains of wildtype yeast cells in the W303 strain background with one copy (LOTOX) and three copies (HITOX) of a galactose inducible construct expressing human alpha-synuclein imaged after 6hours growth in galactose.

FIGURE 3: PMSF and Sulforaphane (SFN) Reduce α-synuclein Aggregation in Yeast

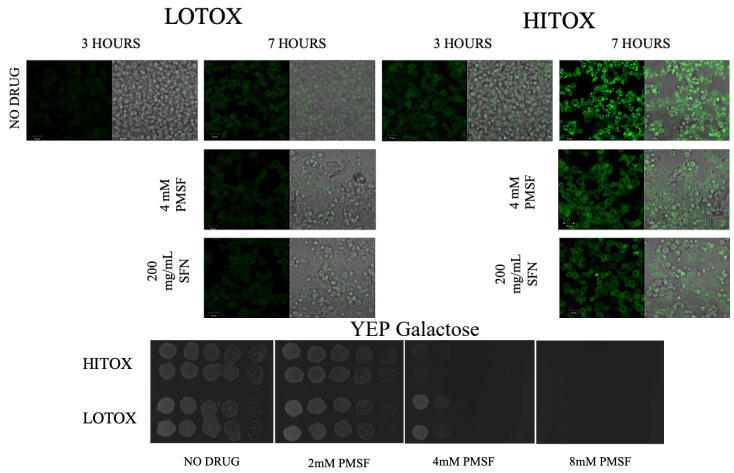
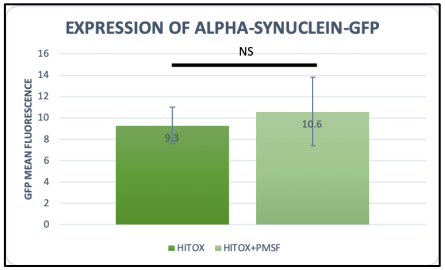


Figure 3: Two strains of *Saccharomyces cerevisiae* were used in this study, 4791 and 4795. 4791 is a high-tox strain expressing 3x α-Synuclein, whereas low-tox is expressing 1x α-Synuclein. The figure above shows that the galactose promoter was turned on at T=3. At T=3, separate cell cultures were given drugs. Aggregate reduction is clear in the pictures at T=7 when compared to the control. In this experiment, both cell strains were grown for 12hrs in YEP glucose liquid media, washed and transferred to YEP raffinose for 3 hours. In the induction step, cultures were washed and inoculated into YEP galactose.

FIGURE 4: PMSF and SFN Do Not Alter α-synuclein-GFP Protein Levels in Hitox Cells

(A) PMSF Does Not Reduce Alpha-Synuclein-GFP Levels in Hitox Cells



(B) SFN Does Not Reduce Alpha-Synuclein-GFP Levels in Hitox Cells

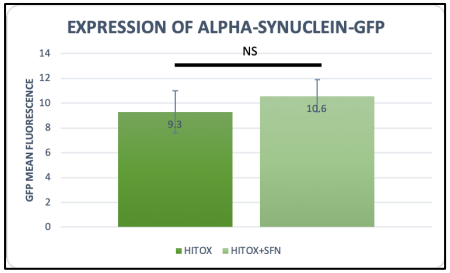


FIGURE 4: Hitox was grown for approximately 12 hours in YPD liquid media, washed, and resuspended in YEP Raffinose in order to prep the galactose promoter. After 3 hours, the cultures were washed and resuspended into YEP Galactose for 3 hours, after which drugs were introduced. For both figures (A) and (B), the P value proved to be insignificant. This shows that neither PMSF nor SFN had an affect on GFP intensity.

CONCLUSIONS

- α-Synuclein aggregates and is toxic in budding yeast.
- PMSF and SFN appear to alleviate protein aggregation.

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Tardiff, D. F., Jui, N. T., Khurana, V., Tambe, M. A., Thompson, M. L., Chung, C. Y., . . . Lindquist, S. (2013). *Yeast reveal a "druggable" Rsp5/Nedd4 network that ameliorates α–Synuclein toxicity in neurons*doi:10.1126/science.1245321

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