Yeast? Yes! A New Trend in Modeling Parkinson's Disease

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By the end of this decade, the baby boomer generation will be progressing well into their 60s. As this aging population rises, so, too, will the incidence of age-related neurodegenerative diseases. The demand for geriatric care will begin to rise, putting a strain on healthcare for the elderly, space available in care facilities, and family members. These diseases such as Alzheimer's, Parkinson's, and amyotrophic lateral sclerosis (ALS or Lou Gherig's Disease), are still all incurable and fatal diseases. Parkinson's Disease (PD) is caused by the death of a specific set of midbrain neurons that control voluntary movement. The accumulation of a misfolded form of the α-synuclein protein into structures called Lewy Bodies may be one toxic agent involved in the pathogenesis of the disease. Four exciting new studies have modeled the misfolding of α-synuclein in yeast. Examining this protein in other cellular systems may shed light on its toxic effects, bringing researchers closer to finding a cure.

In order to gain more insight into α-synuclein's role in PD, the protein has been expressed in several model organisms, such as *Drosophila*, *C. elegans*, and mice (1). One recent model used for α-synuclein expression has been the simple, easily manipulated, eukaryotic organism, yeast. Although yeast cells are vastly different from brain cells, there are actually many similarities in the way both eukaryotic cells conduct protein folding process and the way they maintain quality control over their own proteins. Outeiro et al. first reported α-synuclein expression in baker's yeast in 2003 (2), and the organism has since proven to be a widely used expression system. Three different laboratories have published their findings on α-synuclein expression in yeast in the two years thereafter. In comparing the most recent studies, much *in vivo* evidence is revealed about the protein's lipid binding abilities, aggregation, and toxicity.

Identifying α-synuclein's localization in a eukaryotic cell such as yeast may suggest how the protein interacts in other eukaryotic cells, specifically neurons. Perhaps the most consistent finding among the studies has been the way in which α-synuclein localizes within baker's yeast. Each group tagged α-synuclein to a green fluorescent protein (GFP) in order to visualize the localization of the protein within the cells. They observed that wild-type (WT) α-synuclein and the A53T familial mutant localized to the plasma membrane, while the A30P familial mutant was diffuse in the cytoplasm (3,4,5). This finding is consistent with a number of studies that have reported the protein's lipid and membrane binding abilities. One group reported that a double A30P/A53T mutant displayed both membrane localization and diffuse cytoplasmic fluorescence (4). Two of these yeast studies found that by increasing expression time (3,5), the WT form of the protein lost membrane localization and accumulated within the cytoplasm.

Determining the nature of this α-synuclein aggregation may give insight as to how the protein is involved in the pathogenesis of PD, a notion that is still not completely understood. *In vitro* studies have shown that α-synuclein aggregation is a nucleation-dependent process (6); that is, the protein will aggregate further if a seed of the protein is first formed. This seed formation, first described for an aggregation-prone amyloid bacterial protein in an Alzheimer's study, is dependent upon 1) the initial concentration of the protein, and 2) the amount of time the protein is present in the system (7). To test this hypothesis for α-synuclein *in vivo*, GFP-tagged α-synuclein was expressed in fission yeast, a system with a set of vectors that produce various amounts of the protein. A very different phenotype from baker's yeast was observed in fission yeast: there was no α-synuclein localization to the plasma membrane of fission yeast at any time point during its expression. WT and the A53T mutant formed foci within the cytoplasm, while A30P and the double mutant remained diffuse. Furthermore, by increasing expression time *or* by increasing protein expression levels, WT and A53T α-synuclein displayed greater foci formation. This data is the first *in vivo* study that supports a time *and* concentration dependent process of α-synuclein aggregation (8).

One of the baker's yeast studies also revealed that α-synuclein aggregation is a nucleation-elongation process (5). This study showed that when A30P, which did not form inclusions upon expression by itself, formed aggregates upon coexpression with WT α-synuclein. Zabrocki et al. suggest that the A30P mutant forms inclusions on the nuclei of WT α-synuclein, and that on its own, A30P is defective in nucleation. This supports previous in vitro studies which demonstrate that A30P does not aggregate as easily as other forms of α-synuclein.

Whether or not aggregated α-synuclein causes the neuronal death in Parkinson's Disease patients is still a debated issue in the field. Each study conducted growth analyses to determine if α-synuclein was toxic to yeast cells in order to shed light on the problem. Only one of the three baker's yeast studies found that α-synuclein, by itself, was toxic to wild type yeast strains (3). WT and A53T α-synuclein was toxic when expressed at increased levels, whereas A30P α-synuclein was not. The fission yeast study did not find α-synuclein to be toxic at any level of expression (8). All of the baker's yeast studies, however, found that the protein was toxic if the yeast were compromised in certain ways that may be similar to how neurons are compromised in PD patients. For example, one study revealed that when α-synuclein was coexpressed with the Alzheimer's protein, tau, the combination was toxic to yeast (5); this is a lethal combination in some forms of PD and several other neurodegenerative conditions (9). Another group found that the expression of α-synuclein in yeast cells lacking an antioxidant enzyme, manganese superoxide dismutase, caused toxicity, and more so when cells were treated with hydrogen peroxide (4). This is consistent with the theory that oxidative stress conditions in midbrain neurons contribute to the onset of PD. Two studies expressed α-synuclein in yeast cells with proteasomal mutations; the proteasome is a structure that degrades α-synuclein, and if it were dysfunctional, the build-up of possibly toxic α-synuclein species might occur. Moreover, a defective proteasome has been linked to the pathogenesis of PD as one inherited form of the disease is caused by a mutation in the proteasomal pathway (10). These two studies, however, observed different results: Dixon et al. found α-synuclein to be toxic to cells a 20S proteasome barrel mutation (3), while Sharma et al. only found delayed α-synuclein synthesis and membrane localization (4).

The most consistent findings among all these studies are that A30P is expressed diffusely in the cytoplasm and that α-synuclein localizes to the plasma membrane of

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Table 1: Summary of the four studies' notable findings.
Although variation exists among these studies, it is important to note the consistency of plasma membrane localization and the A30P phenotype.

	Dixon et al. Baker's Yeast Model	Sharma et al. Baker's Yeast Model	Zabrocki et al. Baker's Yeast Model	Brandis et al. Fission Yeast Model
α-Syuclein localizes to the plasma membrane	Yes	Yes	Yes	No
α-Synuclein is toxic to yeast	Wild Type and A53T α-synuclein are toxic to yeast	No	No	No
Evidence of nucleation polymerization of α-synuclein	No	No	Yes	Yes
A30P α-synuclein is diffuse in the cytoplasm	Yes	Yes	Yes	Yes

baker's yeast (see table). Only one study, however, showed that α-synuclein was toxic to yeast when expressed on its own. Although many other assays were conducted in these studies and although variation exists among these studies' findings (see table), the results are consistent with many theories in the field, indicating that yeast may be a good model organism for examining α-synuclein activity. Moreover, a knockout for every gene in the baker's yeast genome exists, which allows for quick evaluation of genes that may protect against α-synuclein toxicity. A study preliminarily conducted this evaluation and revealed eighty-six knockout strains that were toxic upon α-synuclein protein expression (11). Further evaluation of these baker's yeast knockout strains may reveal much more about α-synuclein's cellular activity—information that can then be applied to its activity in neurons. In addition, the fission yeast model for α-synuclein expression provides a good model for α-synuclein misfolding and aggregation. Although fission yeast has not been as widely used to model neurodegenerative diseases, it may in the future serve as an even better tool to study α-synuclein misfolding when more knockout strains become available. These studies on yeast may promote the organism's use for the modeling of other protein misfolding, neurodegenerative diseases—a group of diseases that plague, unfortunately, a significant portion of the world's aging population.

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