

# Components Enhancing $\alpha$ -synuclein Aggregation and Toxicity in a Humanized Yeast

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## Summary

We developed a humanized yeast model to investigate the pathogenic mechanisms of  $\alpha$ -synuclein ( $\alpha$ SYN). Our data demonstrate that  $\alpha$ SYN aggregation is a nucleation-elongation process initiated at the plasma membrane. It can be enhanced by treatment with DMSO and even drugs that influence autophagic and proteasomal clearance have dramatic effects. Moreover, aggregation of  $\alpha$ SYN interferes with endocytosis. Co-expression of  $\alpha$ SYN and protein tau is synergistically toxic for yeast cells and this led us to use our yeast model to screen a human hippocampus cDNA library to identify novel components that enhance toxicity of  $\alpha$ SYN in yeast.

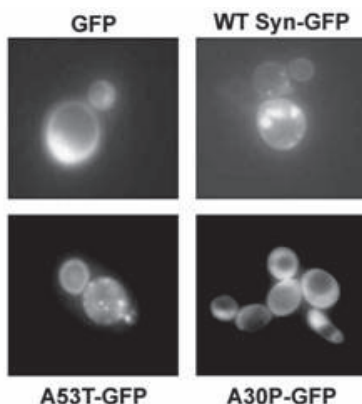
## Introduction

Aberrant aggregation of specific proteins is a common hallmark for neurodegenerative disorders. Parkinson's disease (PD) is marked by fibrillary cytoplasmic inclusions, Lewy bodies, in dopaminergic neurons. Lewy bodies mainly contain aSYN, a presynaptic protein of 140 amino acids presumed to function in vesicle binding. Three  $\alpha$ SYN mutations, A30P, E46K and A53T are associated with early onset familial PD, but they differ in physical properties and neurotoxicity. The etiology of the sporadic form of PD remains unclear, but some highlight the importance of phosphorylation of  $\alpha$ SYN (Bennett, 2005). We used the yeast *S. cerevisiae* to study aggregation of  $\alpha$ SYN *in vivo* and al-

lowed us to confirm and establish fundamental aspects related to  $\alpha$ SYN pathogenesis.

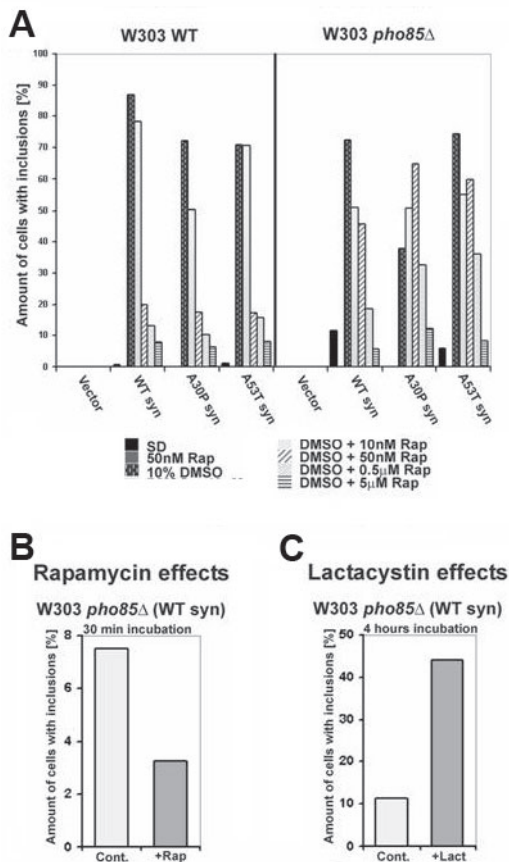
## Results

We expressed conditionally wild-type  $\alpha$ -synuclein (wt- $\alpha$ SYN) or the mutant A30P or A53T, either as native protein or as C-terminal EGFP fusion protein, in the W303-1A yeast strain. None of these proteins induced toxicity in our yeast strains. The expression levels of wt- $\alpha$ SYN and the A53T mutant were moderate and comparable, while much higher expression levels were found for the A30P mutant. Shortly after induction, both wt- $\alpha$ SYN and the A53T mutant were localized at the plasma membrane (Fig. 1) and started to form small inclusions, which often transformed into larger cytoplasmic aggregates in late exponential growth phase. In contrast, the A30P mutant was exclusively located in the cytoplasm and did not give rise to any inclusions (Fig. 1). These data indicated that the interaction with membranes could be important for  $\alpha$ SYN aggregation. Consistently, treatment of the yeast cells with 10% DMSO, which is known to stimulate lipid biosynthesis (Murata *et al.*, 2003), increased aggregation, even in cells expressing the A30P mutant (Fig. 2A). Also treatment of the yeast cells with 50  $\mu$ M lactacystin, a proteasome inhibitor, enhanced the formation of wt- $\alpha$ SYN inclusions, indicating that the proteasome is actively involved in  $\alpha$ SYN turnover in yeast cells (Fig. 2C). On the other hand, treatment of the yeast cells with 50 nM rapamycin, which stimulates autophagy (Noda *et al.*, 2002),



*Figure 1:  $\alpha$ -Synuclein forms inclusions in yeast cells. W303-1A yeast cells transformed with a construct to express  $\alpha$ -synuclein as EGFP fusion protein were grown till mid-exponential phase and analyzed by fluorescence microscopy. Cells transformed with wt- $\alpha$ SYN and A53T mutant showed membrane localized inclusions in contrast to cells expressing the A30P mutant.*

Figure 2:  $\alpha$ -Synuclein aggregates proportional to the lipid content and is eliminated by proteasomal degradation and autophagy. (A) The number of cells that formed inclusions of wt- $\alpha$ SYN or the mutant proteins A30P and A53T increases dramatically when the lipid content of cells was boosted by addition of 10% DMSO to the culture. This effect is counteracted in a dose-dependent manner by simultaneous treatment with rapamycin, which is known to stimulate autophagy (B) Treatment with 50 nM rapamycin for 30 min also reduces the number of *pho85* $\Delta$  yeast cells with inclusion of wt- $\alpha$ SYN. (C) Inhibition of the proteasome by treatment with 50  $\mu$ M lactacystin for 4h enhances the number of *pho85* $\Delta$  yeast cells that formed inclusions of wt- $\alpha$ SYN.



reduced the formation of inclusions (Fig. 2B) and almost completely annihilated the effect of DMSO in a concentration dependent manner (Fig. 2A).

Overexpression of wt- $\alpha$ SYN or the A53T mutant in wild type cells affected endocytosis and led to accumulation of the dye FM4-64 in intermediate vesicles (Fig. 3). This effect was aggravated upon treatment with 10% DMSO. Notably, the inclusions of wt- $\alpha$ SYN or the A53T mutant often colocalized with the FM4 64 stained vesicles. Overexpression of the A30P mutant, however, did not affect endocytosis but when its aggregation was induced by treatment with 10% DMSO, it caused retardation of endosomal transport.

Reminiscent to double transgenic mice (Giasson *et al.*, 2003), co-expression of  $\alpha$ SYN and protein tau enhanced toxicity in yeast as illustrated by a significant growth reduction (Fig. 4A) This effect was most

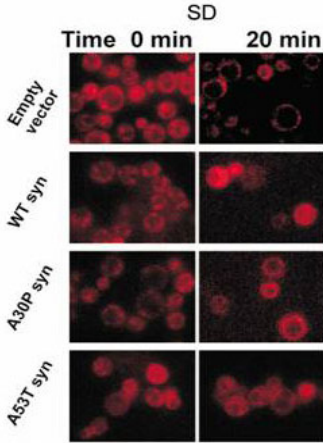


Figure 3: Aggregation of  $\alpha$ -synuclein retards endocytosis. Wild type cells overexpressing wt- $\alpha$ SYN or the mutant proteins A30P and A53T were grown till mid-exponential phase and stained with FM4-64. Endocytosis of the dye was followed until the control strain, transformed with an empty plasmid, and the strain expressing A30P showed staining of the vacuolar membrane.

pronounced when expression of wt- $\alpha$ SYN or A53T was combined with expression of the mutant protein P301L tau (Goedert, 2004) in cells lacking Pho85, the orthologue of human cdk5, a kinase known to play a central role in neurodegeneration (Shelton and Johnson, 2004). To investigate whether this toxicity was related to increased  $\alpha$ SYN aggregation, we co-expressed  $\alpha$ SYN-EGFP fusion proteins with tau proteins. The number of cells with aggregates of wt- $\alpha$ SYN or the A53T mutant in-

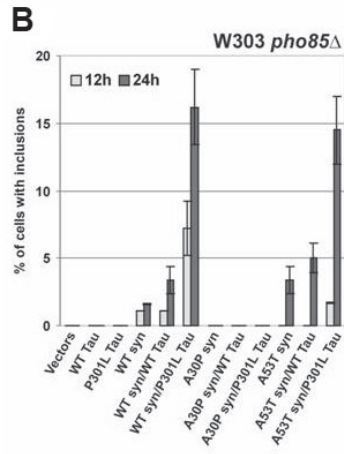
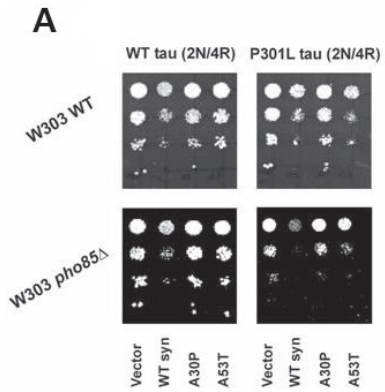


Figure 4: Co-expression of  $\alpha$ -synuclein and protein tau is toxic for yeast cells. (A) Equal amounts of wild type cells and pho85 $\Delta$  mutant cells expressing wild type (WT) or mutant (P301L) human tau, alone or in combination with wt- $\alpha$ SYN or the mutant proteins A30P or A53T, were spotted in serial dilutions on selective agar medium and allowed to grow for 72h. (B) Cells expressing wild type (WT) or mutant (P301L) human tau, alone or in combination with EGFP-fusions of either wt- $\alpha$ SYN, A30P or A53T, were grown for 12h or 24h and the percentage of cells with inclusions of EGFP- $\alpha$ -synuclein was determined. The graph presents data from three independent experiments.

creased dramatically when co-expressed with wt-tau or P301L-tau (Fig. 4B).

A wild-type yeast strain with wt- $\alpha$ SYN overexpression was screened with a human hippocampus cDNA library. Selection was based on growth reduction, similar to the phenotype obtained with co-expression of  $\alpha$ SYN and protein tau (Zabrocki *et al.*, 2005). This screening led to the isolation of 12 human cDNA's that caused significant growth retardation when co-expressed with wt- $\alpha$ SYN in yeast. Further research will deal with the effects of overexpression of these cDNA's in a mammalian model system.

## Conclusions

The data demonstrate that our humanized yeast model recapitulates major aspects of a-synuclein aggregation and cytotoxicity. Therefore, this system offers great potential for defining the underlying mechanisms of toxicity of a-synuclein and its synergistic action when co-expressed with other human proteins.

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