Eukaryon, Vol. 1, 109-115, January 2005, Lake Forest College

Primary Article

Evaluation of STP2-dependent α-Synuclein Toxicity in Yeast: Role of GAPDH?

Arun George Paul* and Ruja Shrestha
Department of Biology
Lake Forest College
Lake Forest, Illinois 60045

Summary

Parkinson disease (PD) is caused by α-synuclein misfolding in the substantia nigra par compacta cells of the brain. A recent study (Willingham et. al., 2003) demonstrated that over expression of wild type (WT) ∝-synuclein in STP2 deletion (STP2Δ) *S. cerevisiae* strains is toxic. Surprisingly, we did not observe any toxicity in 4741 (parent strain) and STP2Δ strains over expressing WT ∝-synuclein or its mutant forms. Moreover, GFP microscopy of α-synuclein in STP2Δ strains showed phenotypes observed in strains with STP2. We observed halos for strains over expressing WT ∝-synuclein and the A53T mutant. ∝-synuclein was cytoplasmic for strains with the A30P mutant and the A30P/A53T double mutant. Western analysis demonstrated that neither the levels of ∝-synuclein expression nor the levels of GAPDH, a pro-apoptotic protein changed in STP2Δ strains. Furthermore, genetic deletion of GAPDH did not show any enhanced growth in strains that overexpressed ∝-synuclein. The lack of ∝-synuclein toxicity in STP2Δ in our study may be due to our use of a different overexpression system, which did not yield amounts high enough to overwhelm protein quality-control systems and yield the toxicity that underlies PD pathogenesis (Berke, 2003) of the cell.

Introduction

Described first by James Parkinson (1817), PD is one of the most common neurodegenerative diseases in the world and affects about 4 million people worldwide (Dauer, 2003). Clinically, the major symptoms are tremor at rest, slowness of movement (bradykinesia), reduced facial expressions, and balance problems (Lang et. al., 1998). Pathologically, PD is caused [1]by the neurodegeneration of the substantia nigra compacta (SNC), resulting in the loss of dopaminergic neurons (Colcher A., 1999). The major clinical hallmark is the presence of eosinophilic inclusions known as Lewy Bodies (LBs) that forms an array of radiating fibrilar structures (Spillantini et. al., 1998). Ultra structural analysis had revealed that LBs are amyloid like fibrils consisting of misfolded ∝-synuclein proteins (Spillantini et. al., 1998). One can acquire PD by either inheriting the particular genetic mutation or sporadically. In familial forms, the misfolding of ∝-synuclein is associated with the substitution of the 53rd Threonine (Polymeropoulos et. al., 1997) and the 30th Proline (Kruger, 1998) by an alanine.

The normal function of ∝-synuclein is still not well known. But evidences do support their role in membrane plasticity (Clayton et. al., 1998). The stabilization of its secondary structure upon binding to synthetic membranes (Davidson et al., 1998), the interaction of ∝-synuclein and phospholipids (Perrin et. al., 2000), and the membrane binding characteristics of ∝-synuclein in cultured cells (Cole et. al., 2002) suggests its role in the plasma membrane. Most recent studies have discovered the lipid-binding domain of ∝-synuclein and propose its roles in the regulation of dopamine transporter activity (Wersinger et. al., 2003).

In 2003, Willingham et. al investigated the different upstream and downstream factors linked with ∝-synuclein. They showed 86 yeast genes that enhanced ∝-synuclein and huntingtin toxicity in their absence. Among them, one was STP2. However, the study did not shed further light on the connections between ∝-synuclein, huntingtin and STP2. Structurally, STP2 or N-hydroxyarylamine sulfotransferase 2 belongs to the family of phenol preferring sulfrotransferases (Khan et. al., 1995). Nevertheless, recent studies have reported its role along with STP1 (AndAndreasson, 2002) as a transcription factor for amino acid permease genes (de Boer et. al., 2000) that transport extra-cellular amino acids to the cell (Nielsen et. al., 2001). Most recent study by Eckert-Boulet et. al., (2004) further expanded the role of STP2. Transcriptional profiling of STP2/STP1 knockout strains of *S. cerevisiae* using DNA micro array showed the up regulation of several carbohydrate metabolic proteins such as glyceraldehydes-3-phosphodehydrogenase (GAPDH3 or Hsp35) and glucokinase (Eckert-Boulet et. al., 2004).

GAPDH3 is one among the three isoforms of GAPDH protein, which is an enzyme that converts glyceraldehyde-3-phosphate to 1,3-biphosphoglycerate in the step 6 of glycolysis (McAlister, 1985). Recent studies have revealed the diverse roles of GAPDH in the neurodegeneration of dopaminerigc neurons (Fukuhara et. al., 2001; Mazzola, 2002). Treating neurodegenerative cells with known GAPDH inhibitors such as CGP 3466B (Waldmeier, 2000), rasagiline (Maruyama, 2001), and deprenyl & propargylamines (Tatton, 2003) have shown rescuing effects. These evidences suggest that GAPDH may play a role in the signal transduction pathways that control neuronal apoptosis (Tatton (2), 2003). Among the three isoforms of GAPDH, studies have also shown the link between GAPDH3 or Hsp35 and protein misfolding during stress (Delgado, 2003). A specific connection between ∝-synuclein and GAPDH was shown by the demonstration of the localization of GAPDH with the LBs of PD affected patients (Tatton et. al., 2000).

Our study aims to investigate the connection between ∝-synuclein and STP2. Based on the link between STP2 and GAPDH (Eckert-Boulet et. al., 2004), we hypothesize that the enhancement of ∝-synuclein toxicity in STP2Δ strains, as demonstrated by Willingham et. al., (2003) was due to elevated GAPDH levels. We examined the toxicity of parent and STP2Δ strains over expressing WT ∝-synuclein. Furthermore, we extended the study by over expressing the mutant forms of ∝-synuclein too. To investigate the role of GAPDH in the toxicity observed by Willingham et. al., (2003), we examined the expression levels of GAPDH in STP2Δ strains. Lastly, to investigate the possibility of enhanced growth by GAPDH inhibition, we examined GAPDHD over expressing WT ∝-synuclein and its mutant forms.

Results

∝-Synuclein toxicity is not enhanced by STP2 Deletion

* This paper was written based on original scholarship conducted in BIO324 Advanced Cell Biology, by Dr. Shubik DebBurman.

We first asked if WT ∝-synuclein toxicity was enhanced in the absence of STP2. Surprisingly, the growth curves of parent and STP2Δ strains over expressing WT ∝-synuclein do not demonstrate any toxicity (Figure 1). For further confirmation, we conducted a yeast viability assay (serial spotting) and still did not observe any toxicity as shown in Fig. 2. We then repeated the experiments on parent and STP2Δ strains over expressing the familial mutants of ∝-synuclein-A30P & A53T, and an artificial double mutant A30P/A53T. However, both assays (Fig. 1 & Fig. 2) demonstrated the absence of any toxicity in any of the STP2Δ strains. Our controls were strains with the parent plasmid (pYES2) and GFP alone.

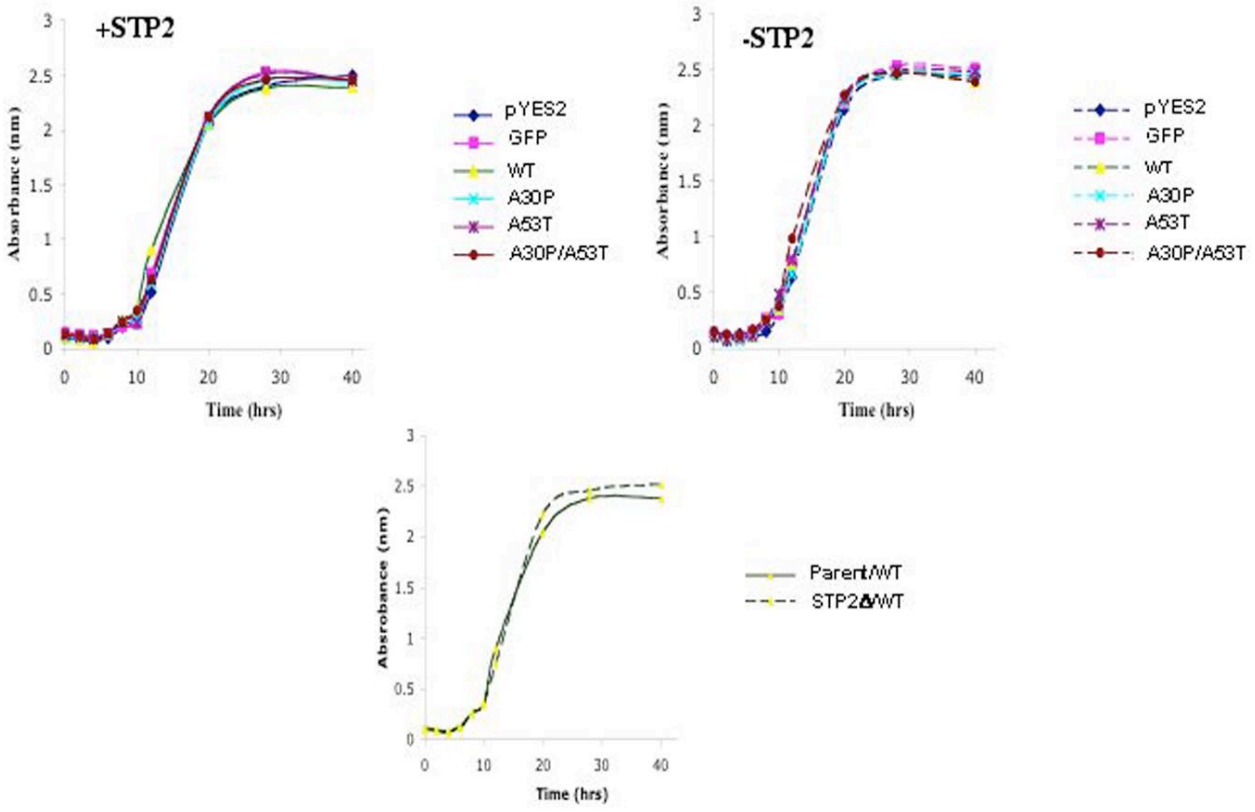

Figure 1. Growth Curves of parent and STP2D Strains
Yeast cells were grown in SC-URA/Galactose for α-Synuclein expression. Absorbance of the growing culture at 0hrs, 3hrs, 6hrs, 9hrs, 12hrs, 18hrs, 24hrs, and 36hrs were taken using the Hitachi U-200 spectrophotometer at 600nm. The growth curves of 4741(left, lines) and STP2D (right, dashed lines) strains shows the absence of any toxicity. Both graphs were plotted to the same scale as demonstrated by the graph in the bottom.

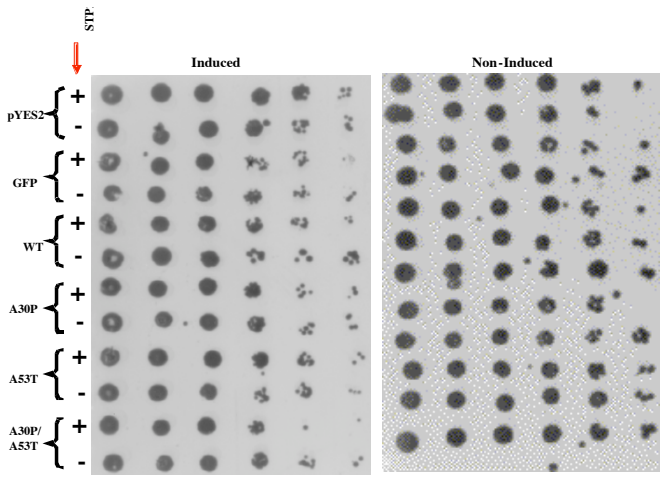

Figure 2. Serial Spotting of Parent and STP2D Strains

Yeast cells were grown to mid-log phase in SC-URA raffinose. They were then normalized to equal densities (2*107 cells/ml), serially diluted (five fold), and spotted on SC-URA glucose (left) and galactose (right) plates. The assay indicates the absence of toxicity in both parent and STP2D strains over expressing WT ∝-synuclein, A30P, A53T, and A30P/A53T. pYES2 and GFP alone were used as controls.

∝-Synuclein expression and size unchanged in STP2Δ strains

To investigate the possibilities of ∝-synuclein aggregation and differences in ∝-synuclein expression, we examined the lystaes of parent and STP2Δ strains by Western analysis. The immunoblotting shows no signal for the parent vector pYES2 and the expected band of size 34 kDA for GFP alone (Sharma, 2004). Equal amount of ∝-synuclein expression was observed for the parent and STP2Δ strains over expressing WT ∝-synuclein and its mutant forms (Figure 3). The minor differences between the different strains could be accounted by the differences in the protein content as demonstrated by coomassie staining (Figure 3). The immunoblotting also did not show ∝-synuclein aggregates (Figure 3), since the bands were of expected size with ~62kDA (Sharma, 2004). We also did not see any differences in either expression level or in the size of ∝-synuclein in strains over expressing the mutant forms of ∝-synuclein (Figure 3).

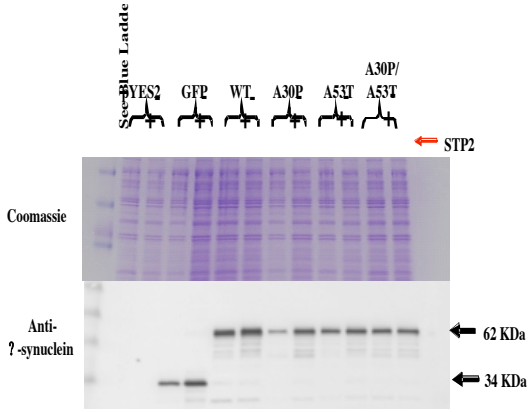

Figure 3. Western Analysis of Parent and STP2D Lysates
Yeast cells (2.5x107 cells/ml) were washed in 50mM Tris (pH 7.5), 10mM Nan3 and solubilized in Electrophoresis Sample Buffer (ESB; Burke, 2000). Samples (25 ul) were run on pre-cast 10-20% Tris-Glycine SDS gels (Invitrogen), using See Blue (15 ul) as the molecular standard (Invitrogen). Gels were transferred to PVDF membranes (BioExpress) and probed with the antibodies: anti-α-synuclein (Santa Cruz, Biotech). To detect the antibodies, the blot was treated with color development solution. WT ∝-synuclein, A30P, A53T, and A30P/A53T migrated to 62 KDA and GFP alone untill 34 KDA. The blot shows the absence of difference in neither size or expression levels of ∝-synuclein.

Cytoplasmic and peripheral localization of ∝-synuclein unvaried in STP2Δ

GFP microscopy was conducted at the 24th hour after induction on all the parent and STP2Δ strains to examine the phenotypes of ∝-synuclein expression (Rows 1 & 2; Figure 4). Strains with the parent plasmid and GFP alone were used as controls (Columns 1 & 2; Figure 4). To evaluate phenotypic changes with time, we examined the strains at the 48th hour also (Rows 2, 3, 5 & 6; Figure 4). Extensive

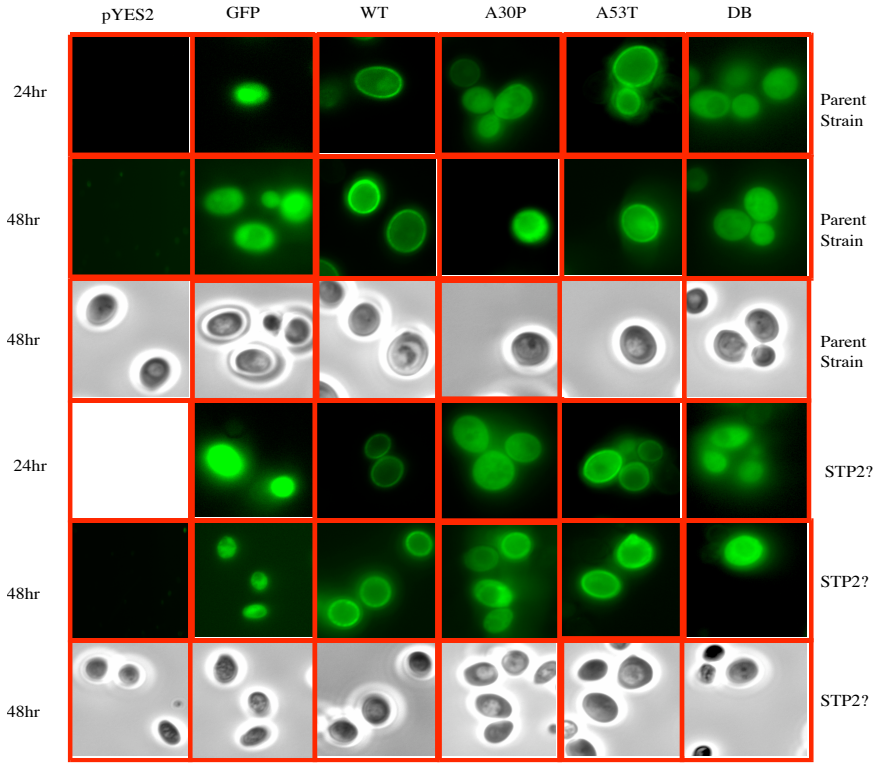

Figure 4. GFP Analysis of Parent and STP2D Strains

To analyze cells under fluorescent microscopy, expression was induced for 24 hours and was diluted to a density of 5*106 cells/ml in 25ml of SC-URA/Galactose media. Cells weregrwon to log-phase, and resuspended in 250ul of SC-URA/Glucose media to concentrate them 100-fold. The figure shows intense cytoplasmic localization for all the strains with A30P mutant and the double mutant A30P/A53T and bright halos for all the strains with WT ∝-synuclein and the A53T mutant. No phenotypic difference was observed at 48hrs after induction.

cytoplasmic localization of ∝-synuclein was observed in the parent and STP2Δ strains over expressing GFP alone, WT ∝-synuclein and the A30P mutant (Columns 2, 3, and 4; Figure 4). However, halos could be seen for strains over expressing A53T and A30P/A53T suggesting the localization of ∝-synuclein in the plasma membrane (Columns 5 and 6; Figure 4). Among the different phenotypes we investigated, intense cytoplasmic localization of ∝-synuclein in the form of spherical bodies also known as foci was seen in the strains over expressing the WT ∝-synuclein and the A53T mutant (Figure 5; top). Quantification of cells with foci at the 24th and 48th hours shows that the % of foci decreases for the parent strain over expressing WT ∝-synuclein and increased for the A53T mutant (Figure 5; bottom). An increase in the % of foci was also seen in the STP2Δ strains overexpressing WT ∝-synuclein and the A53T mutant (Figure 5; bottom).

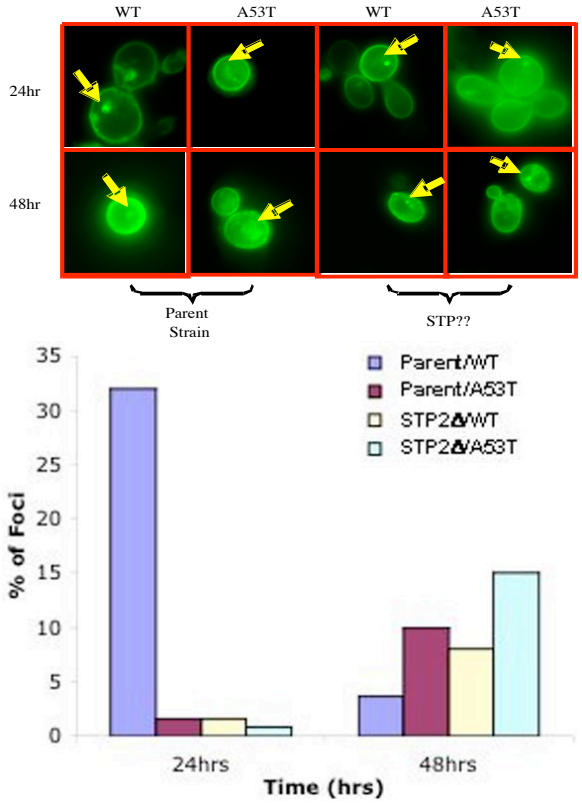

Figure 5. GFP Analysis Reveals Foci
The cells were prepared as in Fig. 4. Foci were observed in in all the strains overexpressing WT and A53T ∝-synuclein. Quantification was carried out by counting cells in the number of foci in six adjacent fields. The number of foci decreased for parent strain with WT ∝-synuclein at the 48th hour, but increased for the STP2D strains with WT ∝-synuclein and A53T mutant (bottom). An increase in the % of foci was also seen in the parent strain with the A53T mutant (bottom).

GAPDH Levels Remains Unchanged in STP2Δ strains

We hypothesized that the enhanced toxicity observed by Willingham et. al., (2003) was due to elevated levels of GAPDH. To test this hypothesis, we investigated the expression levels of GAPDH by Western analysis on the cell lysates of parent and STP2Δ strains over expressing WT ∝-synuclein (Figure 6). The immunoblotting shows the GAPDH band with expected size of 43.5Kda. However, we observed the absence of any difference in GAPDH expression between the parent and STP2Δ strains. We also did not see any difference between the parent and STP2Δ strains over expressing the mutant forms of ∝-synuclein--A30P & A53T and the A30P/A53T. The cell lysates of strain with the parent plasmid and GFP alone were used as controls.

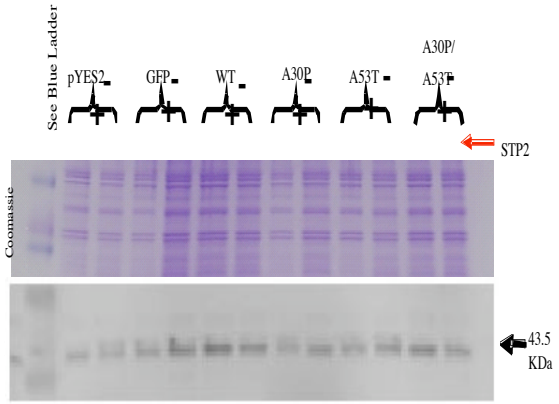

Figure 6. Western Analysis of Parent and STP2D Lysates Examining GAPDH Expression
The Western was conducted as described in Figure 3. The blot was probed with the primary anti- GAPDH antibody (ChemiCon International) followed by the secondary anti-PGK (Molecular Probes) antibody. As expected, the GAPDH protein migrated till 43.5 KDA. The blot shows the absence of difference in GAPDH expression between Parent and STP2D strains.

Deletion of GAPDH does not enhance growth

Although we did not observe any toxicity in STP2Δ strains, we asked whether the deletion of GAPDH might enhance growth in a STP2 independent manner. To investigate this possibility, we used a strain lacking GAPDH3. We compared the growth of parent and GAPDHΔ strains over expressing WT ∝-synuclein and it mutant forms by serial spotting (Figure 7). Further extending the study with GAPDHD over expressing A30P, A53T, and A30P/A53T also did not shown any enhancement in growth. The strains with the parent plasmid and GFP alone were again used as controls (Figure 7).

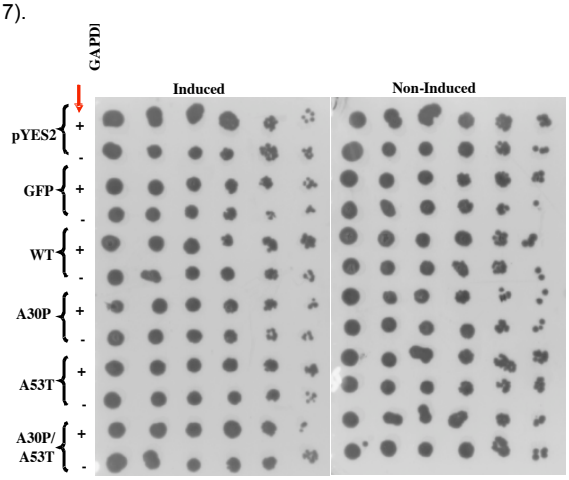

Figure 7. Serial Spotting of Parent and GAPDHD Strains
The spotting was conducted as described in Figure. 2. The assay indicates the absence of any toxicity in both parent and GAPDHD

strainsover expressing WT ∝-synuclein, A30P, A53T, and A30P/A53T. pYES 2and GFP alone were used as controls.

Discussion

In this study, we investigated the enhanced ∝-synuclein toxicity observed by Willingham et. al., (2003) in STP2 deletion strains of *S. cerevisiae* and extended this analysis to familial mutants. We proposed this enhanced toxicity was due to increased GAPDH levels. However, we neither observed toxicity nor difference in GAPDH levels in STP2Δ strains.

STP2 toxicity may require higher expression of _-synuclein

Our study demonstrated the absence of toxicity in STP2Δ strains through OD_{600} and spotting assays. This is paradoxical to the results of Willingham *et. al*., (2003). Besides, we did not see toxicity in the parent strain too. This is also contradictory. Because, other recent studies in yeast (Outeiro et. al., 2003) and other model systems such as mice have demonstrated over expression of ∝-synuclein to be toxic (Yamada, 2004). The absence of the toxicity should not be because of the strains; since both Willingham et. al., (2003) and us used the 4741 based STP2 deletions and galactose based ∝-synuclein induction systems. However, the vectors used by Willingham et. al., (2003) was p426, where as we used the pYES2 vector, which posses minor differences in the sequence. This might affect the galactose based induction system to such an extent that the optimal amount of ∝-synuclein necessary for being toxic in STP2Δ background may not be expressed. Optimal amount of misfolded proteins is a crucial factor for toxicity or aggregate formation (Outerio et. al., 2003). Because, if the optimal amount of ∝-synuclein is not present, the protein quality-control system of cells, including uniquitin ligase (Kitada, 1998), Hsp 70 (Hartl, 2002), and uniquitin C-terminal hydrolase (Leroy, 1998; Liu, 2002) may cope up with misfolded ∝-synuclein (Berke, 2003; Taylor, 2002). To investigate the effect of mutant ∝-synuclein in inducing toxicity, we extended the study by over expressing the mutant variants of ∝-synuclein--A30P & A53T, and the artificial double mutant A30P/A53T. Similar to the over expression of WT ∝-synuclein, the mutant forms are also shown to be lethal (Outerio et. al., 2003). However, we did not see any difference in the growth rate of the parent and STP2Δ strains. This might also be accounted by the difference in the vector system we used for over expressing ∝-synuclein.

STP2 does not appear to influence *in vivo* and *in vitro* a-synuclein aggregation

We next examined the presence of any abnormal phenotypes in parent and STP2Δ strains by GFP microscopy. Between the two types, we did not see any difference in the phenotypes (Fig. 4) thereby showing the neutral effect of STP2 deletion. Previous studies have shown that ∝-synuclein is a plasma membrane specific protein (Wersinger et. al., 2003). The parent and STP2Δ strains over expressing WT ∝-synuclein and it mutant form A53T showed halos suggesting the localization of ∝-synuclein in the plasma membrane. This indicates that the A53T mutation is neutral to the plasma binding of ∝-synuclein. The cytoplasmic localization of ∝-synuclein in the plasma membrane for parent and STP2Δ strains with the A30P mutant and the double mutant A30P/A53T suggests the necessity of the 30th proline for plasma membrane binding. Our results agree with other studies that have conducted similar experiments (Outerio et. al., 2003; Wang et. al., 2004). Evaluation of any variation in the dominant phenotype at the 48th hour did not demonstrate any phenotypic differences. This suggests the absence of the progress of any lethal or unusually phenotypes that are undetectable at the 24th hour.

Although the dominant phenotypes were plasma membrane and cytoplasmic localization, we also observed foci in the cytoplasam of parent and STP2Δ strains over expressing WT, and A53T. However, the percentage of foci was very low. This further indicates the absence of the optimal amount of ∝-synuclein required for being toxic, if foci are the cause of toxicity in the study by Willingham *et. al*., (2003). Because, simply doubling the amount of ∝-synuclein is shown to change the membrane localization of ∝-synuclein to cytoplasmic foci (Outerio et. al., 2003). Quantification of this at the 24th and 48th hours showed a decrease in the number of foci for parent strain with WT ∝-synuclein and an increase for STP2Δ with WT ∝-synuclein and A53T mutant. An increase in foci was also seen for parent strain with A53T mutant. The question of whether the foci are aggregates of ∝-synuclein remains to be answered. However, the absence of toxicity highlights the hypothesis of the 'Protective Function of Aggregates' (Goldberg, 2000). Whether the foci are ∝-synuclein aggregates or not, the decrease in foci for parent strain with WT suggests the activation of chaperone systems that removes them (Hartl, 2002). However, a definitive pattern cannot be formulated, since the number of foci is increasing for STP2Δ with WT ∝-synuclein and its mutant form A53T and also for parent strain with A53T mutant. To make further conclusions, the experiment needs to be repeated. But it suggests the existence of a molecular overlap in the signal transduction pathways of the three types of strains that shown an increase in foci formation at the 48th hour.

Although, aggregation is not a prerequisite for ∝-synuclein toxicity (Goldberg, 2000), it is possible that the toxicity demonstrated by Willingham et. al., (2003) was due to ∝-synuclein aggregation. Besides, in vitro studies by Conway et. al., (1998) have shown that ∝-synuclein mutations A30P and A53T accelerate the oligomerization of ∝-synuclein. Recent studies on yeast have also shown difference in the expression levels of ∝-synuclein with A30P mutant having elevated expression over WT and A53T (Outerio et. al., 2003). We investigated aggregate formation by immunoblotting for ∝-synuclein on the cells lysates prepared from parent and STP2Δ strains. However, we did not see any difference on the size and on the expression level of ∝-synuclein. The absence of aggregates or multimers might also be because of the absence of adequate amount of ∝-synuclein necessary for fibrilization due to the induction system we used.

Relevance of GAPDH

Based on Eckert-Boulet et. al., (2004), we proposed that the enhanced toxicity in STP2Δ (Willingham et. al., 2003) was due to GAPDH over expression. However, immunoblotting demonstrates the absence of any difference in the expression level of GAPDH between the parent and STP2Δ strains. The absence of the difference might be because of the difference in the strains used. Eckert-Boulet et. al., (2004) used the yeast strain *gap/ura3* M4054, where as we used 4741. Or else it might be because of the high signal-to-noise ratio of the DNA Micro array system used by Eckert-Boulet *et al*., (2004) leading to a false positive signal for GAPDH.

Our initial hypothesis was the role of GAPDH in STP2 dependent ∝-synuclein toxicity was demonstrated to be invalid. Therefore, we proposed that deletion of GAPDH3 might enhance the growth in both parent and STP2D strains, if ∝-synuclein toxicity is independent of STP2 yet dependent on GAPDH. Similar studies in mice had resulted in the rescue of dopaminergic neurons undergoing apoptosis (Fukuhara et. al., 2001). However, we did not demonstrate any enhanced growth in both parent and GAPDHΔ strains. This could be explained by the fact that there might not have been any toxicity in the first place because of the absence of optimal amount of ∝-synuclein necessary for being toxic. Or

else, GAPDH might not have any link between apoptosis or ∝-synuclein in *S. cerevisiae*.

Conclusion

We conclude that the absence of enhanced STP2 dependent ∝-synuclein toxicity in our study was due to the difference in the vector used. Our results propose a model where the pYES2 based induction systems expresses ∝-synuclein below the optimal amount that is required to bypass the protein quality -controlled systems of the cell

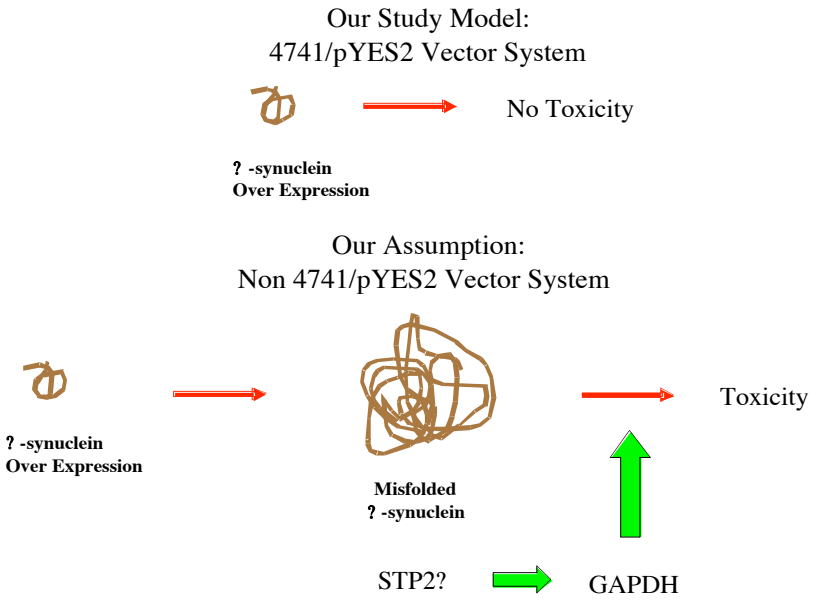

Figure 8. Molecular Model that Links STP2, ∝-synuclein and GAPDH
We predict that the absence of STP2 dependent ∝-synuclein toxicity as shown by Willingham *et. al*., (2003) in our study was due to the difference in the vector system used. In our system (4741/pYES2), ∝-synuclein expression was not adequate enough toby pass the protein quality-control systems in the cell. Also, the GAPDH expression levels did not change in our STP2D strains, which was paradoxiacal to the observation of Eckert-Boulet et. al., (2004). We assume this difference was because of the difference in the yeast strain used.

(Figure 8). Therefore, to completely verify the role of GAPDH in STP2 dependent ∝-synuclein toxicity, the study needs to be repeated with other vectors.

Experimental Procedures

α-Synuclein Constructs
Human wild type and A53T mutant α-synuclein cDNAs were a gift from Christopher Ross (Johns Hopkins University). A30P and A30P/A53T mutant α-synuclein were created from wild type and A53T mutant α-synuclein, respectively, using site-directed mutagenesis (Invitrogen). Wild type and mutant α-synuclein cDNAs were sub cloned into the pYES2.1/V5-His- TOPO yeast expression vector (Invitrogen). All α-synuclein forms were also tagged with GFP using a two-step cloning strategy. α-Synuclein cDNAs were first sub cloned into mammalian expression vectors, pcDNA3.1/C-terminal GFP and pcDNA3.1/N-terminal GFP (Invitrogen). α-Synuclein-GFP-CT fusion genes were then amplified and subcloned into yeast expression vector, pYES2.1. Chemically competent *E. coli* were transformed with the α-synuclein and α-synuclein-GFP expression plasmids and grown on LB-ampicillin media for selection. All constructs were verified by DNA sequencing. The parent pYES2.1 vector (Invitrogen) and GFP in pYES2.1 vector were used as controls.

Expression in Yeast Strains
α-Synuclein expression plasmids were transformed as described (Burke, 2000) into URA-3-deficient, STP2-deficient, and GAPDH3-deficient *S. cerevisiae* strains 4741, 6603, and 4422 respectively. For selection, yeast cells were grown on synthetic-complete media lacking uracil (SC-URA). Presence of α-synuclein constructs was confirmed by polymerase-chain reaction. The pYES2.1 vector, containing a galactose inducible promoter (GAL1), allowed for regulated α-synuclein expression. Yeast cells were first grown overnight in SC-URA glucose (2%) media at 30°C. Cells were washed with water and diluted to log-phase in SC-URA galactose (2%) media to induce expression.

Western Analyses

Yeast cells ($2.5x10^7$ cells/ml) were washed in 50mM Tris (pH 7.5), 10mM Nan_3 and solubilized in Electrophoresis Sample Buffer (ESB; Burke, 2000) containing 2% SDS, 80mMTris (ph 6.8), 10% glycerol, 1.5% DTT, 1mg/ml bromophenol blue, and a cocktail of protease inhibitors and solubilizing agents (1% Triton-X 100, 1mM PMSF, 1mM benzamidine, 1mM sodium orthovanadate, 0.7 μg/ml pepstatin A, 0.5 μg/ml leupeptin, 10μg/ml E64, 2μg/ml aprotinin, and 2μg/ml chymostatin). Samples (20-30 ul) were run on pre-cast 10-20% Tris-Glycine SDS gels (Invitrogen), using See Blue (15 ul) as the molecular standard (Invitrogen). Gels were transferred to PVDF membranes (BioExpress) for 120 minutes at 30V and probed with the antibodies: anti-α-synuclein (Santa Cruz, Biotech), anti- GAPDH antibody (ChemiCon International) and anti-PGK (Molecular Probes); secondary antibody for anti-GAPDH antibody. Antibodies were diluted in blocking buffer as recommended. To detect the antibodies, the following color development solution was used: 100ml Tris Buffer (pH 9.5) containing 15mg/ml BCIP and 30mg/ml of NBT dissolved in 100% and 70% dimethylformamide, respectively (BioRad).

Table 1. Different α-synuclein Constructs and the Respective Strains Used

Construct	Strain	S. cerevisiae Strain Used
pYES2	Parent	4741
GFP (CT)	Parent	4741
WT-α-synuclein-GFP (CT)	Parent	4741
A30P α-synuclein-GFP (CT)	Parent	4741
A53T α-synuclein-GFP (CT)	Parent	4741
A30P/A53T α-synuclein-GFP (CT)	Parent	4741
pYES2	STP2Δ	6603
GFP (CT)	STP2Δ	6603
WT-α-synuclein-GFP (CT)	STP2Δ	6603
A30P α-synuclein-GFP (CT)	STP2Δ	6603
A53T α-synuclein-GFP (CT)	STP2Δ	6603
A30P/A53T α-synuclein-GFP (CT)	STP2Δ	6603
pYES2	GAPDHΔ	4422
GFP (CT)	GAPDHΔ	4422
WT-α-synuclein-GFP (CT)	GAPDHΔ	4422
A30P α-synuclein-GFP (CT)	GAPDHΔ	4422
A53T α-synuclein-GFP (CT)	GAPDHΔ	4422

A30P/A53T α-synuclein-GFP (CT)	GAPDHΔ	4422

Growth Analyses

For OD_{600} analysis, yeast cells were grown overnight in SC-URA/Glucose and were washed the next day twice with water and transferred into two sets of cultures (SC-URA/Glucose and SC-URA/Galactose) at early log-phase (5*106 cell/ml). The absorbance readings of yeast cells expressing α-synuclein at 0hrs, 3hrs, 6hrs, 9hrs, 12hrs, 18hrs, 24hrs, and 36hrs were taken at 600nm using the Hitachi U-200 spectrophotometer. For spotting, yeast cells were grown to mid-log phase in SC-URA raffinose (2%), normalized to equal densities (2*107 cells/ml), serially diluted (five-fold), and spotted on SC-URA glucose (2%) or galactose (2%) plates. Photographs were scanned after 2-3 days of growth.

GFP Microscopy and Quantification

To analyze cells under fluorescent microscopy, expression was induced for 24 hours as described previously and was diluted to a density of 5*106 cells/ml in 25ml of SC-URA/Galactose media and allowed to grow into log phase. Cells were then washed twice with deionized water and resuspended in 250ul of SC-URA/Glucose media to concentrate them 100-fold. 10 ul of the cells was added to a slide and viewed under the Zeiss Axiovert-100 fluorescent microscope and images were acquired under blue-filter settings using the Metamorph 4.0 imaging software. Quantification was carried out by counting cells in the number of each type of phenotype in six adjacent fields and calculating the ratio of cells displaying certain patterns: general fluorescence (cytoplasmic localization), bright halos (peripheral localization), and foci (possible aggregation) to the total number of cells in the six fields together.

Acknowledgements

We wish to thank Dr. Shubhik K. DebBurman, Sara Herrera, Jessica Price, and Katrina Brandis of Lake Forest College who helped in this project. This work was supported by a grant from the National Science Foundation and National Institutes of Health.

References

Andreasson C and Ljungdahl PO. 2002. Receptor-mediated endoproteolytic activation of two transcription factors in yeast. *Genes Dev.,* 24, 3158.

Berke, Sarah J Shoesmith and Henry L Paulson. 2003. Protein aggregation and the ubiquitin proteasome pathway: gaining the UPPer hand on neurodegeneration. *Current Opinion in Genetics & Development, 13, 253.*

Burk SL, Campbell, Jones KA, and Shulman RG. 1987. 31P NMR saturation-transfer measurments in *Saccharomyces cerevisiae*: characterization of phosphate exchange reactions by idoacetate and antimycin A inhibition. *Biochemistry*, 17, 7483.

Clayton, David F and Juila M. George. 1998. The synucleins: a family of proteins involved in synaptic function, plasticity, neurodegeneration and disease. *TINS*, 21, 249.

Colcher A, Simuni T. 1999. Clinical manifestations of Parkinson's disease. *Medical Clinics of North America*, 83(2): 327.

Cole, Nelson B, Diane D. Murphy, Theresa Grider, Susan Rueter, Dawn Brasaemle, and Robert L. Nussbaum. 2002. Lipid Droplet Binding and Oligomerization Properties of the Parkinson's Diease Protein ∝-synuclein. *The Journal of Biological Chemistry*, 277, 6344.

Conway, Kelly A., James D. Harepr, and Peter T. Lansbury. 1998. Accelerated in vitro fibril formation by a mutant ∝-synuclein linked to early on-set Parkinson's Disease. *Nature Medicine*, 4: 1318.

Davidson, W. Sean, Ana Jonas, David F. Clayton, and Julia M. George. 1998. Stabilization of ∝-synuclein secondary structure upon binding to synthetic memberanes. *The Journal of Biological Chemistry*, 273, 9443.

de Boer M, Nielsen PS, Bebelman JP, Heerikhuizen H, Andersen HA, and Planta RJ. Stp1P, Stp2P, and Abfip are involved in regulation of expression of the amino acid transporter gene BAP3 of Saccharomyces cerevisiae. *Nucleic Acids Res.*, 28, 974.

Delgado ML, Gil ML, Gozalbo D. 2003. Starvation and temperature upshift cause an increase in the enzymatically active cell wall-associated glyceraldehyde-3-phosphate dehydrogenase protein in yeast. *FEMS Yeast*, 3, 297.

Eckert-Boulet N, Nielsen PS, Friis C, dos Santos MM, Nielsen J, Kielland-Brandt MC, and Regenberg B. 2004. Transcriptional profiling of extracellular amino acid sensing in *Saccharomyces cerevisiae* and the role of stp2 and stp2. *Yeast*, 21, 635.

Fukuhara Y, Takeshima T, Kashiwaya Y, Shimoda K, Ishitani R, Nakashima K. 2001. GAPDH knockdown rescues mesencephalic dopaminergic neurons from MPP+ -induced apoptosis. Neuroreport. 12, 2049.

Goldberg, Mathew S and Peter T. Lansbury Jr. 2000. Is there a cause-and-effect relationship between ∝-synuclein fibrilization and Parkinson's disease? *Nat Cell Biol*., 7, 115.

Hartl, F. Ulrich and Manajit Hayer-Harrtl. 2002. Molecular Chaperons in the Cytosol: from Nascent Chain to Folded Protein. *Science*, 295, 1852.

Khan AS, Taylor BR, Filie JD, Ringer DP, and Kozak CA. 1995. Rat phenol-preferring sulfotransferase genes (Stp1 and Stp2): localization to mouse chromosomes 7 and 17. *Genomics*, 26, 417.

Kitada T, Asakawa S, Hattori N, Matsumine H, Yamamura Y, Minoshima S, Yokochi M, Mizuno Y, Shimizu N.1998. Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature*, 392, 605.

Kruger, Rejko, Wilfried Kuhn, Thomas Muller, Dirk Woitalla, Manuel Graeber, Sigfried Kosel, Horst Przuntek, Jorg T. Epplen, Ludger Schols, and Olaf Riess. 1998. Ala30Pro mutation in the gene encoding ∝-synuclein in Parkinson's disease. *Nature Genetics*, 18, 106.

Lang AE, Lozano AM (1998). Parkinson's disease: First of two parts. *New England Journal of Medicine*, 339(15): 1044.

Leroy, Elisabeth, Rebecca Boyer, Georg Auburger, Barbara Leube, Gudrun Ulm, Eva Mezey, Gyongyi Harta, Michael J. Brownstein, Sobhanaddítya Jonnalagada, Tanya Chernova, Anindya Dehejia, Christian Lavedan, Thomas Gasser, Peter J. Steinbach, Keith D. Wilkinson, Mihael H. Polymeropoulos. 1998. The ubiquitin pathway in Parkinson's disease. *Nature,* 395, 451.

Liu, Yichin, Lara Fallon, Hilal A. Lashuel, Zhihua Liu, and Peter T. Lansbury. 2002. The UCH-L1 Gene Encodes Two Opposing Enzymatic Activities that Affect Éø-Synuclein Degradation and Parkinson's Disease Susceptibility. *Cell*,111, 209.

Maruyama W, Akao Y, Youdim MB, Davis BA, Naoi M. 2001. Transfection-enforced Bcl-2 overexpression and an anti-Parkinson drug, rasagiline, prevent nuclear accumulation of glyceraldehyde-3-phosphate dehydrogenase induced by an endogenous dopaminergic neurotoxin, N-methyl(R)salsolinol. *J Neurochem*., 78, 727.

Mazzola JL, Sirover MA. 2002. Alteration of intracellular structure and function of glyceraldehyde-3-phosphate dehydrogenase: a common phenotype of neurodegenerative disorders? *Neurotoxicology*., 23, 603.

McAlister, Lee and Michael J. Holland. 1985. Differential expression of the three yeast glyceraldehydes-3-phosphate dehydrogenase gens. *The Journal of Biological Chemistry*, 260, 15019.

Outerio, Tiagao Fleming and Susan Lindquist. 2003. Yeast Cells Provide Insight into Alpha-Synuclein Biology and Pathology. *Science*, 302, 1772.

Parkinson, James. 1817. An Essay on the Shaking Palsy. Whittingham & Rowland, London, 1817.

Perrin, Richard J., Wendy S. Woods, David F. Clayton, and Julia George. 2000. Interaction of human ∝-synuclein and Parkinson's disease Nariants with Phospholipids. *The Journal of Biological Chemistry*, 275, 34393.

Polymeropoulos, Mihael H., Christian Lavedan, Elisabeth Leroy, Susan E. Ide, Anindya Dehejia, Amalia Dutra, Brian Pike, Holly Root, Jeffrey Rubenstein, Rebecca Boyer, Edward S. Stenroos, Settara Chandrasekharappa, Aglaia Athanassiadou, Theodore Papapetropoulos, William G. Johnson, Alice M. Lazzarini, Roger C. Duvoison, Giuseppe Di Iorio, Lawrence I. Golbe, and Robert L. Nussbaum. 1997. Mutation in the ∝-synuclein gene Identified in Families with Parkinson's Disease. *Science*, 276, 2045.

Sharma, Nijee. 2004. Baker's Yeast Promotes Understanding of ∝-synuclein's Functional and Pathogenic Roles. Lake Forest college, IL-60045.

Spillantini, Maria Grazia, Marie Luise SCHMIDT, Virginia M.-Y. Lee, John Q. Trojanowski. 1997. ∝-synuclein in Lewy Bodies. *Nature*, 388, 839.

Tatton NA. 2000. Increased caspase 3 and Bax immunoreactivity accompany nuclear GAPDH translocation and neuronal apoptosis in Parkinson's disease. *Exp Neurol*, 166, 29.

Tatton W, Chalmers-Redman R, Tatton N. 2003. Neuroprotection by deprenyl and other propargylamines: glyceraldehyde-3-phosphate dehydrogenase rather than monoamine oxidase B. *J Neural Transm*., 110, 509.

Tatton WG, Chalmer-Redman R, Brown D, Tatton N. 2003. Apoptosis in Parkinson's disease: signals for neuronal degradation. *Ann Neurol.,* 53, 61.

Taylor, J. Paul, John Hardy, and Kenneth H. Fischback. 2002. Toxic Proteins in Neurodegerative Disease. *Science*, 296, 1991.

Waldmeier PC, Boulton AA, Cools AR, Kato AC, Tatton WG. 2000. Neurorescuing effects of the GAPDH ligand CGP 3466B. *J Neural Transm Suppl.*, 60, 197.

Wersinger, Christophe, Delphine Prou, Philippe Vernier, Hyman B. Niznik, and Anita Sidhu. 2003. Mutations in the lipid-binding domain of ∝-synuclein confer overlappingg, yet distinct, functional properties in the regulation of dopamine transporter activity. *Molecular and Cellular Neuroscience*, 24, 91.

Willingham, Stephen, Tiago Fleming Outerio, Michael J. Devit, Susan L. Lindquist, and Paul Muchowski. 2003. Yeast Genes that Enhance the Toxicity of a Mutant Huntingtin Fragment or ∝-synuclein. *Science*, 302, 1769.

Yamada M, Iwatsubo T, Mizuno Y, Mochizuki H. 2004. Overexpression of alpha-synuclein in rat substantia nigra results in loss of dopaminergic neurons, phosphorylation of alpha-synuclein and activation of caspase-9: resemblance to pathogenetic changes in Parkinson's disease. *J Neurochem.*,91, 451.