α-Synuclein Alters Proteasome Function, Protein Synthesis, and Stationary Phase Viability*

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α-Synuclein appears to play a role in mediating neurotoxicity in a number of neurodegenerative disorders, collectively referred to as synucleinopathies. Most of these disorders are associated with aging and a probable impairment of the proteasome-proteolytic pathway, although the relationship between aging, proteasome inhibition, and α-synuclein toxicity has not been fully elucidated. Recent studies suggest that yeast may provide a useful system for studying the biology and toxicity of α-synuclein in mitotic cells, recapitulating many features observed in the various synucleinopathy disorders. Additional studies indicate that the stationary phase model of aging in yeast provides a useful system for understanding the biochemistry and regulation of aging in post-mitotic cells. In the present study we examined the effect of wild type and mutant α-synuclein (A30P) on multiple aspects of proteasome homeostasis, protein synthesis, as well as the ability of cells to survive stationary phase aging. These data demonstrate that α-synuclein alters proteasome composition, impairs proteasome-mediated protein degradation, impairs protein synthesis, and impairs the ability of cells to withstand stationary phase aging. Interestingly, α-synuclein had little effect on intracellular proteasome content or protein ubiquitination, and did not increase the vulnerability of cells to a variety of stressors. Together, these data suggest that yeast may be useful for understanding the ability of α-synuclein to impair proteasome-mediated protein degradation, as well as for understanding the basis for age-related α-synuclein cytotoxicity.

α-Synuclein is a protein of as yet unknown function, which is believed to play a role in a wide array of neurodegenerative disorders including Dementia with Lewy bodies, Lewy body variant of Alzheimer disease, and Parkinson disease (1–3). Collectively, these disorders have been referred to as synucleinopathies (4, 5), with the vast majority of synucleinopathies being sporadic in nature. Familial forms of most synucleinopathies have also been documented, with strong experimental and genetic evidence indicating that many familial forms of synucleinopathy disorders are mediated by the presence of mutations in α-synuclein (6, 7). The basis for α-synuclein toxicity has not been fully elucidated, although mutations in α-synuclein are believed to contribute to cellular toxicity by altering the normal structure and/or function of α-synuclein (8–11). In both sporadic and familial forms of the different synucleinopathy disorders age is the biggest risk factor for disease onset, although cellular basis for this observation remains to be elucidated. A variety of in vitro and in vivo models have been developed to understand the molecular and cellular basis of α-synuclein toxicity (12, 13). Together, these studies have implicated a role for α-synuclein-induced alterations in mitochondrial homeostasis, protein aggregation, oxidative stress, and inhibition of proteasome function as mediators of α-synuclein toxicity (10, 14–16).

Recent studies have demonstrated that yeast are a useful model with which to study α-synuclein toxicity (17–19), with the previous studies in mitotic yeast contributing to our understanding of what cellular processes are likely important in promoting and inhibiting α-synuclein toxicity. Numerous studies have demonstrated that yeast are also useful in studying the biochemistry and regulation of aging in both mitotic and post-mitotic cells (20–22). For the analysis of post-mitotic cell aging, stationary phase or chronological yeast aging has been demonstrated to recapitulate multiple features of aging that are present during the aging of post-mitotic mammalian cells (19–21). Additionally, the stationary phase model of aging has allowed for the identification of genetic manipulations that may have beneficial effects toward the aging of mammalian cells (20, 21, 23).

In the present study we conducted experimentation to understand the effects of wild type and mutant α-synuclein toward multiple aspects of cellular homeostasis including proteasome function, protein synthesis, and the ability to survive stationary phase aging. These data demonstrate that α-synuclein has dramatic effects on proteasome-mediated protein degradation, protein synthesis, and impairs the ability of post-mitotic cells to survive stationary phase aging. Together, these data contribute to our understanding of α-synuclein-induced cytotoxicity, and suggest that yeast may be particularly useful for studying the effects of α-synuclein on steady state protein expression, as well as the aging-related cytotoxicity that is observed in a variety of synucleinopathies.

MATERIALS AND METHODS

Transformation and Propagation of Yeast Cultures—Wild type and mutant α-synuclein, as well as full-length human tau, were amplified from plasmids of pcDNA3.1 containing the full-length form of the indicated gene (Gift from Dr. Rodney Guttmann). The resulting PCR fragment, of the correct size, was ligated into pADGal4 to generate the constitutive expression plasmids pAD5syn, pAD5Syn–A30P, or pADtau. These expression plasmids together with empty pADGal4 vector were then transformed (25) into yeast strain BY4742 obtained from the American Tissue Culture Collection (Y201389). The stably transformed colonies were then randomly picked up and grown in SD–(LEU) medium to reach stationary phase (26–28). Identical results were obtained
whether cells were maintained in expired medium or water (data not shown).

**Analysis of α-Synuclein Expression**—The yeast cells containing pAD-GαA vector, pAD/Syn, and pAD/Syn-AP were grown in SD (−LEU) medium and 1 ml of each cell culture was harvested, respectively, on days 1 and 7 of stationary phase for RNA preparation. Total yeast RNA was extracted by TRI reagent (Sigma), and 2 μg of total RNA was used in *in vitro* reverse transcription (RT)-PCR by the SuperScript III one-step RT-PCR kit (Invitrogen). The RT-PCR was performed for 30 min at 42 °C followed by 25 repeating cycles of 45 s at 95 °C, 45 s at 55 °C, and 1 min at 72 °C. Primers used for RT-PCR were the same used for synuclein cloning as above. Primers used for 18 S control were: 5′-TTATGACCCACTCGGACC-3′ and 5′-CCATCGGTTGAAACC-GATA-3′. To examine the expression of synuclein, we conducted Western blot analysis as described previously (29, 30). The expression of tau was also confirmed by Western blot analysis. Equal amounts of cellular lysate (50 μg) were separated using a 7.5% SDS-PAGE gel, and probing for synuclein using an anti-synuclein monoclonal antibody or tau antibody (kindly provided by Dr. Rodney Gutmann).

**Analysis of Cellular Viability**—Cell viability was determined by monitoring the ability of cells to support a viable colony. All yeast cells were initially seeded at an OD of 0.1 and grown in the same volume of SD (−LEU) medium. On days 4, 7, 14, and 21, the same volume of yeast cells was diluted 100, 1,000 times. Then the same volume of each diluted cell was collected and subjected to a serial dilution, with 5 μl of yeast cells from each respective dilution loaded onto YPD plates and grown at 30 °C for 2–3 days. Similar results were obtained whether cells were maintained in expired medium or water (data not shown).

**Analysis of the Ability of Cells to Support Viable Colonies following Oxidative Stressors and Inhibitors of Protein Synthesis**—An equal number of pre-cultured yeast cells were transferred to fresh SD (−LEU) medium containing 10 mM sodium selenite (34). Briefly, yeast cells were harvested by centrifugation (2000 × g) were separated using 7.5% SDS-PAGE gel, and probed for ubiquitin using anti-ubiquitin antibodies (Chemicon, StressGen).

**Purification of Proteasome Complex**—Yeast 20 S proteasomes were purified using a modified version of previously published protocols (33, 34). Briefly, yeast cells were harvested by centrifugation (2000 × g for 5 min) and then washed with ice-cold stop buffer (0.9% NaCl, 1 mM NaN₃, 10 mM EDTA, 50 mM NaF). The resulting cell pellet was then resuspended in 20 S proteasome activity buffer (35), and the cells were lysed with acid-washed glass beads. The extract was clarified by centrifugation (10,000 × g for 15 min) and the resulting supernatant was then loaded onto a Hitrap Q HP column (Amersham Biosciences). The column was washed with a 5-fold volume of proteasome activity buffer (35), and then eluted with the same buffer containing 1 M NaCl. The purified proteasome fraction was identified by quantifying the chymotrypsin-like activity of the 20 S proteasome (33, 36) in each of the resulting fractions.

**Analysis of Proteasome Composition, and Quantification of Individual Proteolytic Activities in the Purified Proteasome Complex**—To determine the composition of the proteasome, the proteasome fraction was dialyzed using membrane tubing, and 20 μg of protein from each dialysate were taken for two-dimensional gel electrophoresis. The two-dimensional procedures were performed as described previously by our laboratory (28, 31), following the manufacturer’s recommendations. Briefly, the samples were dissolved in sample buffer and loaded onto 11-cm ReadyStrip IPG strips with a pH range from 4 to 7 for overnight rehydration. The protein were then focused using the following parameters: 200 V for 5 h, 1,000 V for 5 h, and 3,000 V for 12 h. The strips were then equilibrated and run in 8–16% Tris-HCl gels at 200 V for 1 h. The individual spots were detected using a silver staining kit (Amersham Biosciences). The individual chymotrypsin-like, trypsin-like, and post-glutamyl peptidase activities of the purified 20 S proteasome (1 μg) were quantified by monitoring the cleavage of the peptide substrates specific for each individual activity as described previously from our laboratory (35).

**RESULTS**

**Generation of Cells Expressing Wild Type and Mutant α-Synuclein**—In the present study we generated yeast that were stably transformed with either full-length wild type or mutant α-synuclein (Fig. 1A). Cells transformed with wild type or mutant α-synuclein exhibited a similar and significant amount of α-synuclein expression (Fig. 1, B and C), with no expression of α-synuclein observed in cells transformed with empty vector. The expression of either wild type or mutant α-synuclein did not appear to affect cell growth (Fig. 1D), or alter cellular morphology (data not shown), during normal growth conditions. The expression of α-synuclein appeared to be unchanged even at day 7 stationary phase (data not shown).

**Wild Type and Mutant α-Synuclein Alter Proteasome-mediated Protein Degradation**—To elucidate whether α-synuclein alters proteasome-mediated protein degradation, we quantified short-lived protein degradation, which is predominantly mediated by the proteasome (28, 31, 32), in cells stably transformed with α-synuclein. During normal growth conditions there was a significant impairment in proteasome-mediated protein degradation in cells expressing wild type α-synuclein (Fig. 2A), which was even more pronounced in cells expressing mutant α-synuclein (Fig. 2A). Interestingly, cells expressing mutant α-synuclein exhibited a nearly complete inhibition of short-lived protein degradation (Fig. 2A). We next examined proteasome-mediated protein degradation in cells at the earliest stage of stationary phase aging (day 4) (20–23, 28, 31), to elucidate what effect stationary phase aging had on cells expressing α-synuclein. Both wild type and mutant α-synuclein exhibited a nearly complete ablation of short-lived protein degradation (Fig. 2B), whereas short-lived protein degradation in cells expressing empty vector exhibited a preservation of some proteasome activity. To ensure that the observed results were specific for α-synuclein, we conducted identical analysis using cells that have been stably transformed with full-length human tau (a cytoskeletal protein involved in Alzheimer disease), using the same promoter and expression vector as outlined for α-synuclein. In these studies we observed that there was no significant effect of tau expression on protein degradation (day 1 tau expressing cells, 100% ± 4; day 4 tau expressing cells, 92% ± 5, data expressed as percent of control values, mean ± S.E.). These data are consistent with previous studies using reporters for proteasome-mediated protein degradation (17, 38, 39), which demonstrated α-synuclein-inhibited ubiquitin-dependent proteolysis, which was presumed to be primarily degraded by the proteasome.

To determine whether the alterations in short-lived protein degradation were associated with alterations in the amount of protein ubiquitination, we conducted Western blot analysis of protein ubiquitination at days 1 and 4 stationary phase. These studies revealed that there was no significant difference between the amount of protein ubiquitination present in vector and α-synuclein expressing cells under normal growth condi-

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1 The abbreviation used is: RT, reverse transcription.
tions at day 1 (Fig. 2C). The amount of protein ubiquitination was significantly reduced in all cell types at day 4 stationary phase, as compared with normal growth conditions (Fig. 2C). At day 4 stationary phase, α-synuclein expressing cells exhibit a modest increase in protein ubiquitination, evident at a variety of molecular weights, as compared with vector-transformed cells (Fig. 2C). A band corresponding with free ubiquitin revealed that at day 4 stationary phase the amount of free ubiquitin was also increased in cells expressing either wild type or mutant α-synuclein (A30P), as compared with vector-transformed cells. This result was confirmed using multiple anti-ubiquitin antibodies (data not shown).

Wild Type and Mutant α-Synuclein Have Minimal Effects on the Proteasome Complex—We next sought to elucidate whether changes in short-lived protein degradation were possibly because of the fact that α-synuclein caused an alteration in individual proteasome activities, altered the amount of proteasome complex present in cells, or potentially altered the composition of the proteasome complexes. To address this we purified the 20 S proteasome from cells under normal growth conditions, and at day 4 stationary phase. The purified proteasome complex (1 μg) from each cell type and condition was then analyzed for chymotrypsin-like, trypsin-like, or post-glutamyl peptidase activities (Fig. 3, A–C). Cells expressing wild type and mutant α-synuclein exhibited a small but significant decrease in chymotrypsin-like activity, as compared with vector-transformed cells (Fig. 3A). No other proteolytic activity of the proteasome was significantly altered in cells expressing wild type or mutant α-synuclein (Fig. 3, B and C). These data suggest that the specific activity of the proteasome complex is not significantly impaired in cells expressing α-synuclein, but do not rule out the possibility that α-synuclein may decrease the amount of proteasome complexes present in cells. To address this we quantified the amount of proteasome (μg) present in 1 mg of cell lysate. Together, these data demonstrate that under normal growth conditions, as well as day 4 stationary phase, α-synuclein did not have any significant effect on the intracellular proteasome content (Fig. 3B). Lastly, we examined the composition of the 20 S proteasome in cells transformed with empty vector, wild type α-synuclein, and mutant α-synuclein. These studies revealed that both wild type and mutant α-synuclein decreased the incorporation of several proteasome-associated proteins (Fig. 4, A–D), as compared with cells transformed with empty vector. To provide a quantitative assessment of the changes in protein incorporation within proteasome complex, we quantified the expression of individual
protein spots from multiple experiments (Fig. 4D). In these studies, we observed that in α-synuclein expressing cells, both elevations and decreases in multiple proteasome-associated proteins could be observed (Fig. 4D).

Wild Type and Mutant α-Synuclein Alter Protein Synthesis—Because proteasome-mediated protein degradation was so dramatically impaired in cells expressing wild type and mutant α-synuclein, we became interested if there was evidence for a potential negative or inhibitory feedback loop, whereby protein synthesis may also be inhibited in conditions where inhibitions in protein synthesis were observed. Analysis of protein synthesis revealed that both wild type α-synuclein and mutant α-synuclein dramatically impaired protein synthesis, as compared with cells transformed with empty vector, under normal growth conditions (Fig. 5A). A similar degree of inhibition in protein synthesis was also observed during early stationary phase (day 4), in cells expressing wild type and mutant α-synuclein (Fig. 5B).

α-Synuclein Alters Ability of Cells to Survive Stationary Phase Aging—We then sought to elucidate whether the expression of wild type or mutant α-synuclein altered the ability of the cells to survive stationary phase aging. Cells expressing empty vector exhibited an increase in cell death, as evident by impairment in the ability to support a viable colony, during increasing periods of stationary phase (Fig. 6). Cells expressing wild type and mutant α-synuclein exhibited a significantly greater impairment in the ability to survive stationary phase aging, as compared with cells transformed with empty vector (Fig. 6). To confirm that the increase in cytotoxicity was specific for α-synuclein, we conducted stationary phase viability stud-
ies in cells stably transformed with human tau. In these studies we observed that during increasing periods of stationary phase, cells expressing tau exhibited viability that was indistinguishable from vector-transformed cells (Fig. 6), highlighting the specificity of our findings with regards to /H9251-synuclein decreasing stationary phase viability.

We next sought to elucidate whether the decreased viability during stationary phase was specific for cellular aging, or was just an artifact of cells expressing /H9251-synuclein being more vulnerable to all stressors. Cell viability was analyzed following exposure to oxidative stressors and inhibitors of protein synthesis. These stressors were chosen based on the relevance of oxidative stress to cellular aging (23), and the fact that cells expressing /H9251-synuclein exhibited a pre-existing impairment in protein synthesis. Interestingly, under exponential growth conditions, cells expressing wild type and mutant /H9251-synuclein did not exhibit any enhanced toxicity toward oxidative stressors (hydrogen peroxide, menadione) or protein synthesis inhibitors.

**Fig. 3.** Analysis of proteasome activities in purified proteasome complexes, and analysis of intracellular proteasome content, in cells expressing /H9251-synuclein. The amount of chymotrypsin-like (A), trypsin-like (B), and post-glutamyl peptidase activity (C) was quantified using 1 μg of purified 20 S proteasome from empty vector, wild type /H9251-synuclein (Syn), or mutant /H9251-synuclein (A30P) under normal growth conditions (Day 1) or day 4 stationary phase (Day 4). Data are presented as specific activity in nmol⁻¹ × mg⁻¹ × min⁻¹. D, the amount of proteasome complexes purified from 1 mg of the cell lysates from empty vector, wild type /H9251-synuclein (Syn), or mutant /H9251-synuclein (A30P) under normal growth conditions (Day 1) or day 4 stationary phase (Day 4) was calculated. Data are representative of results from two separate experiments. *, p < 0.05 compared with vector-transformed cells.
These data suggest that the toxicity of wild type and mutant α-synuclein may be selective for stationary phase aging.

**DISCUSSION**

In the present study we demonstrate that both wild type and mutant α-synuclein alter multiple aspects of proteasome homeostasis, most notably causing an impairment in proteasome-mediated protein degradation. It is important to note that proteasome-mediated protein degradation was quantified as the percent degradation of the total short-lived protein pool, and was therefore not an artifact of there being decreased protein synthesis. Previous studies have demonstrated that cells expressing α-synuclein exhibit an impairment in their ability to degrade a protein reporter that is commonly utilized for analyzing proteasome-mediated protein degradation (17, 38, 39). This protein reporter contains a degradation signal that marks it for ubiquitin-mediated protein degradation, which is predominantly mediated by the proteasome. Whereas these previous studies have been informative, they do not indicate whether the degradation of most short-lived proteins, which includes a large percentage of non-ubiquitinated proteins, is also altered in cells expressing α-synuclein. In the present study we demonstrate that in cells transformed with α-synuclein there is a gross impairment in the degradation of most short-lived proteins, and therefore our data extends the findings from previous studies, by demonstrating that following expression of α-synuclein in yeast there is a dramatic and pervasive impairment in proteasome-mediated protein degradation. Interestingly, in the present study the amount of ubiquitinated protein did not dramatically increase, even though there was a robust impairment of proteasome-mediated protein degradation. However, the elevation in free ubiquitin within α-synuclein cells at day 4 stationary phase suggests that alterations in the ubiquitin pathway may be occurring during stationary phase. Whether the elevation in free ubiquitin is mediated by impairments in ubiquitination, or conversely is mediated by impairments in de-ubiquitination pathways, is currently being investigated. We also cannot rule out at the present time that the elevated levels of free ubiquitin represent an elevation in heat shock protein response (with ubiquitin being a heat stress response protein), although initial studies indicate that the heat shock protein response is selectively

[Fig. 4. Analysis of proteasome composition. The composition of the 20 S proteasome was quantified using 10 μg of purified 20 S proteasome from empty vector (A), wild type α-synuclein (Syn) (B), or mutant α-synuclein (A30P) (C). The individual protein spots were separated using two-dimensional electrophoresis, and developed using silver stain. D, the intensity of the indicated spots was quantified using PDQuest software. Data are representative of results from two separate experiments.]


In the present study there was an alteration in proteasome composition in cells expressing α-synuclein, which may have significantly contributed to the apparent preservation of peptidase activities within the proteasome complex. Previous studies from our laboratory have demonstrated that oxidative stress can dramatically alter the expression of proteasome subunits in neural cells (35), which may play a role in the preservation of proteasome function during low level oxidative stress. Studies from other laboratories have demonstrated that proteasome composition can dramatically alter susceptibility to oxidant-induced inactivation (47), as well as alter the function of the proteasome complex (48). We found no evidence for the association of α-synuclein with the proteasome complex in the present study (data not shown), even though previous studies have suggested that α-synuclein interacts with the proteasome complex (44). Even though there is great homology between the yeast proteasome and the mammalian proteasome, the lack of association between α-synuclein and the proteasome in the present study may be the result of yeast not expressing all or exactly the same proteasome subunits found in human brain. However, it is important to point out that previous studies that demonstrated such an interaction did not define whether α-synuclein was binding to the free S6 (which is more abundant), or binding to S6 in the mature proteasome complex (44). As such, α-synuclein may be interacting with free proteasome subunits in yeast and human brain, and studies are currently underway to clarify this potentially important issue.

Protein targeting, protein unfolding, and insertion of the protein into the proteasome complex must occur for the proteasome to degrade proteins (49). Because there is no significant alteration in the amount of proteasome complexes, and no dramatic alteration in the amount of peptidase activities within the proteasome complexes was observed, we believe that the impairment in proteasome-mediated protein degradation is mediated by alterations in protein targeting, protein unfolding, and/or protein insertion into the proteasome. It is therefore possible that impairments in heat shock protein response, alterations in cytoplasmic flow, or the development of blockages within the proteasome complex at crucial protein entry sites, may all be responsible for mediating an impairment in proteasome-mediated protein degradation. Interestingly, the impairment in proteasome-mediated proteolysis becomes more severe during stationary phase aging. In the various synucleinopathies there is believed to be an inhibition of proteasome function that is above what is observed during normal aging. We believe that yeast will provide a useful model for understanding how the effects of α-synuclein on the proteasome are exacerbated by aging, and may allow for the identification of the genes that positively and negatively regulate this process.

In the present study we observed that both wild type and mutant α-synuclein inhibited protein synthesis during normal degradation of known proteasome substrates, or assembly of the proteasome complex, in neural cells or transgenic mice (42). This previous study, as well as the data in the present article, may therefore appear to be in direct opposition to previous studies that have reported that α-synuclein impairs peptidase activities associated with the proteasome (43–45). However, it is important to point out that these previous studies measured proteasome function in crude lysates, which measures the activity of free proteasome subunits (which account for up to 2% of the total cellular protein) as well as proteasome complexes (46). Because of this fact it is unclear if a loss of peptidase activity, when measured in crude lysate extracts, represents the loss of peptidase activity within the proteasome complex or whether it indicates that there is an alteration in the activity or amount of free proteasome subunits.

In the present study there was a dramatic alteration in the amount of peptidase activities associated with the proteasome complex (data not shown), even though previous studies have demonstrated such an interaction did not define whether α-synuclein was binding to the free S6 (which is more abundant), or binding to S6 in the mature proteasome complex (44). Although there is great homology between the yeast proteasome and the mammalian proteasome, the lack of association between α-synuclein and the proteasome in the present study may be the result of yeast not expressing all or exactly the same proteasome subunits found in human brain. However, it is important to point out that previous studies that demonstrated such an interaction did not define whether α-synuclein was binding to the free S6 (which is more abundant), or binding to S6 in the mature proteasome complex (44). As such, α-synuclein may be interacting with free proteasome subunits in yeast and human brain, and studies are currently underway to clarify this potentially important issue.

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growth conditions, with mutant α-synuclein even impairing protein synthesis at day 4 stationary phase, when protein synthesis is dramatically decreased for all cell types. To the best of our knowledge, these are the first data to demonstrate the ability of α-synuclein to impair protein synthesis, and could potentially be important to defining a new effect of α-synuclein on neuronal homeostasis. Whereas the basis for the decreased protein synthesis is currently not known, there are two likely candidates. Impairments in protein synthesis may represent a negative feedback as the direct result of impaired proteasome-mediated protein degradation, with cells making less protein as they sense an impairment in the ability to remove proteins from the intracellular environment. Preliminary studies indicate that low-level proteasome inhibition produces a robust impairment in protein synthesis, with protein synthesis inhibitors not impairing proteasome function, which would be consistent with our negative-feedback model (data not shown). Another possibility is that α-synuclein induces endoplasmic reticulum stress, which has been reported previously in neural models of Parkinson disease (50), which then promotes an impairment of protein synthesis. Experiments are currently underway to clarify the basis for the impairment in protein synthesis that is observed in the present study.

The most exciting and surprising finding in the present study was our observation that cells expressing wild type and mutant α-synuclein were unable to survive stationary phase aging to the same extent as vector-transformed cells, with stationary phase toxicity most pronounced in cells expressing mutant α-synuclein. It is important to note that the vulnerability of α-synuclein expressing cells appeared specific for stationary phase aging, as there was no alteration in cell death between empty vector and α-synuclein-transformed cells in response to a variety of stressors. Additionally, because expression of the cytoskeleton protein tau did not alter stationary phase viability, our data suggest that α-synuclein induces a potentially specific and selective susceptibility to the stress of stationary phase aging.

It is interesting to note that in our hands the toxicity of α-synuclein in stationary phase cultures was similar whether cells were maintained in expired medium or water (data not shown). Previous studies have demonstrated that maintaining cells in water can promote different effects than the maintenance of cells in expired medium (21). Whereas we are
unsure of the mechanisms responsible for this observation, it is important to note that cells expressing α-synuclein did not exhibit an enhanced vulnerability to oxidative stressors, even though resistance to oxidative stressors is often a predictor of which cells will be most able to withstand stationary phase aging (21). In our hands similar results for proteolyis, protein synthesis, and stationary phase viability were observed in multiple clones. It is important to note that some of these clones exhibited higher levels of α-synuclein expression (data not shown). The reproducibility of the effects of α-synuclein in the present study, apparently independent of α-synuclein expression levels, suggests that the level of α-synuclein expression may not be a critical factor in the development of age-associated α-synuclein toxicity.

Yeast have many attributes that would be expected to make them useful for studying age-related increases in α-synuclein toxicity (18). Studies have demonstrated that stationary phase aging recapitulates multiple aspects of post-mitotic cell aging in mammalian cells. For example, decreases in proteasome function (28), increased evidence for oxidative stress (decreased glutathione and increased protein oxidation) (51, 52), as well as the accumulation of DNA mutations (24), have all been reported to occur in stationary phase aging. Yeast have been utilized to understand most cellular processes that are important to homeostasis of mammalian cells, they express numerous human homologs to human genes, they can be rapidly transformed to express any human genes of interest, and per-