Yeast Genes That Enhance the Toxicity of a Mutant Huntingtin Fragment or α-Synuclein

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Genome-wide screens were performed in yeast to identify genes that enhance the toxicity of a mutant huntingtin fragment or of α-synuclein. Of 4850 haploid mutants containing deletions of nonessential genes, 52 were identified that were sensitive to a mutant huntingtin fragment, 86 that were sensitive to α-synuclein, and only one mutant that was sensitive to both. Genes that enhanced toxicity of the mutant huntingtin fragment clustered in the functionally related cellular processes of response to stress, protein folding, and ubiquitin-dependent protein catabolism, whereas genes that modified α-synuclein toxicity clustered in the processes of lipid metabolism and vesicle-mediated transport. Genes with human orthologs were overrepresented in our screens, suggesting that we may have discovered conserved and nonoverlapping sets of cell-autonomous genes and pathways that are relevant to Huntington's disease and Parkinson's disease.

Huntington's disease (HD) is a fatal, inherited neurodegenerative disorder that is characterized by disturbances in movement, cognition, and personality. The mutation that causes HD is an expansion of CAG repeats [encoding polyglutamine (polyQ)] in the gene *IT-15* (which encodes the huntingtin protein) (*1*). Parkinson's disease (PD) is a major neurodegenerative disorder characterized by muscle rigidity, bradykinesia, resting tremor, and postural instability (*2*). Although the vast majority of cases of PD are idiopathic, a small percentage are caused by missense mutations of the α-synuclein gene (*3*, *4*). One neuropathological feature shared by HD and PD is the occurrence of ubiquitinated intraneuronal inclusion bodies in diseased brains. Huntingtin, and/or truncation products of huntingtin, are the major components of cytoplasmic and nuclear inclusion bodies observed in HD, and α-synuclein is the major component of inclusion bodies (Lewy bodies) in PD. Huntingtin (*5*) and α-synuclein (*6*) assemble into fibrillar protein aggregates that display many properties of amyloid in vitro and in vivo. The precise roles of protein aggregation, amyloid formation, and inclusion bodies in HD, PD, and other amyloid diseases remain controversial, and it is not clear whether common pathogenic mechanisms occur in these disorders.

Here, we have used the baker's yeast *Saccharomyces cerevisiae* as a model eukaryotic organism to test the hypothesis that the downstream targets and molecular mechanisms by which a mutant huntingtin fragment and α-synuclein mediate toxicity are distinct. Similar to neurons, yeast transformed with mutant huntingtin fragments form inclusion bodies in a process regulated by yeast homologs of Hsp40 and Hsp70 (*7*, *8*). As in many types of mammalian cells, overexpression of mutant huntingtin fragments in yeast has no effect on cell viability. This feature allows genetic screens to be performed to identify genes that unmask, or enhance, toxicity. Here, we have used a yeast gene deletion set (YGDS) of 4850 viable mutant haploid strains (*9*, *10*) to identify genes that enhance toxicity of a mutant huntingtin fragment or α-synuclein.

In our model of huntingtin inclusion body formation in *S. cerevisiae* (*8*), yeast are transformed with constructs that express exon 1 of the huntingtin gene with a normal (HD20Q) and expanded (HD53Q) polyQ repeat under control of the *CUP1* promoter. The aggregation and inclusion body–forming properties of huntingtin fragments with expanded polyQ tracts can be reproduced faithfully in *S. cerevisiae* (*8*). Similarly, overexpression of wild-type or mutant (A53T) human α-synuclein in yeast results in the formation of cytoplasmic inclusion bodies that, at the level of light microscopy, are similar to those formed by mutant huntingtin fragments in yeast (*11*).

A collection of 4850 yeast strains was transformed with constructs that express HD53Q or α-synuclein under the control of inducible promoters and was plated onto selective media in the absence of induction (*12*). Mutants that were sensitive to a HD53Q or α-synuclein were isolated by replica plating onto media that contained the appropriate inducer of protein expression. We confirmed putative HD53Q- or α-synuclein–sensitive mutants by retesting isolated colonies in spotting assays that measure cell viability (Fig. 1). Although positive colonies were selected originally because of their complete lack of growth (synthetic lethality) after induction, the retests indicated that a sublethal effect on toxicity (synthetic sickness) occurred in

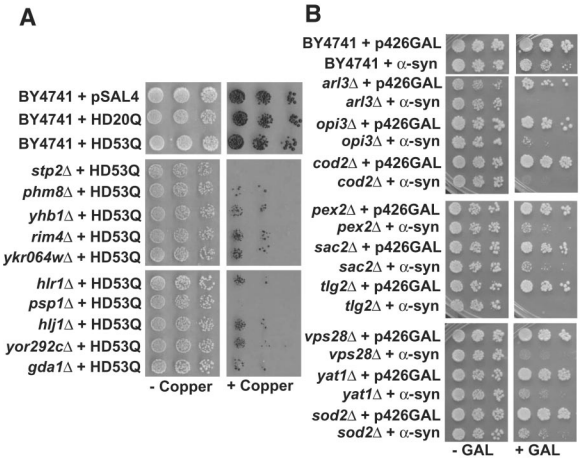

Fig. 1. Expression of a mutant huntingtin fragment (HD53Q) or α-synuclein causes synthetic sickness or lethality in yeast gene deletion strains. (**A**) Yeast cells transformed with empty vector (pSAL4), HD20Q, or HD53Q were grown in liquid synthetic complete medium lacking uracil (SC-URA) to log phase and then induced for 24 hours in SC-URA + copper. Samples of cells were removed from liquid cultures before (T = 0) and after (T = 24 hours) copper induction, were spotted on plates containing SC-URA ± 400 μM copper, and were incubated at 30°C for 3 days. Shown are fivefold serial dilutions starting with equal numbers of cells. Spotting assays derived from single transformants for 10 unique deletion strains and the parental control strain (BY4741) are shown. (**B**) Yeast cells transformed with empty vector (p426GAL) or α-synuclein (α-syn) were grown in liquid synthetic complete medium lacking uracil (SC-URA + glucose) to log phase and then induced for 6 hours in SC-URA + galactose. Samples of cells were removed from liquid cultures before (T = 0) and after (T = 6 hours) galactose induction, were spotted on plates containing SC-URA ± galactose, and were incubated at 30°C for 3 days. Shown are fivefold serial dilutions starting with equal numbers of cells. Spotting assays derived from single transformants for nine unique deletion strains and the parental control strain (BY4741) are shown.

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many of the deletion strains (Fig. 1). Of 4850 mutants, 52 (~1%) were identified with enhanced toxicity of HD53Q, and 86 (~2%) with enhanced toxicity of wild-type α-synuclein (Tables 1 and 2). Although overexpression of α-synuclein caused a modest decrease in cell viability in wild-type yeast (BY4741), all mutant strains that enhanced α-synuclein toxicity had a phenotype that was more severe than that observed in wild-type yeast (Fig. 1B).

Of the HD53Q-sensitive mutants, 77% (40/52) corresponded to genes for which a function or genetic role has been determined experimentally or can be predicted (Table 1) (*13*). Thirty-five percent (14/40) of these genes clustered in the functionally related categories of response to stress, protein folding, and ubiquitin-dependent protein catabolism, based on annotations in the Yeast Proteome Database (Fig. 2) (*14*). The remaining genes were dispersed among numerous and diverse functional categories (Fig. 2). Fifty-two percent (27/52) of the genes we identified are annotated as having human orthologs (Table 1), a value that is significantly higher than the percentage of genes in the yeast genome with mammalian orthologs (~31%, $P \leq 1 \times 10^{-10}$) (*15*).

We next characterized the effects of HD20Q overexpression in yeast gene deletion strains sensitive to HD53Q. About 77% (40/52) of the deletion strains transformed with HD20Q displayed wild-type viability or exhibited a slight decrease in cell viability (table S1). Although 23% (12/52) of strains transformed with HD20Q displayed a noticeable loss of viability, in each case the phenotype was equivalent to or less severe than that observed with HD53Q (table S1). HD53Q-induced synthetic sickness or lethality observed in yeast gene deletion strains was partially rescued by overexpressing human orthologs (*FKBP2*, *GSS*, and *DNAJA2*) of several yeast genes identified in the screen (*FPR2*, *GSH2*, and *HLJ1*, respectively) (fig. S1). To determine whether a correlation exists between HD53Q aggregation and toxicity in the yeast gene deletion strains, filter-trap

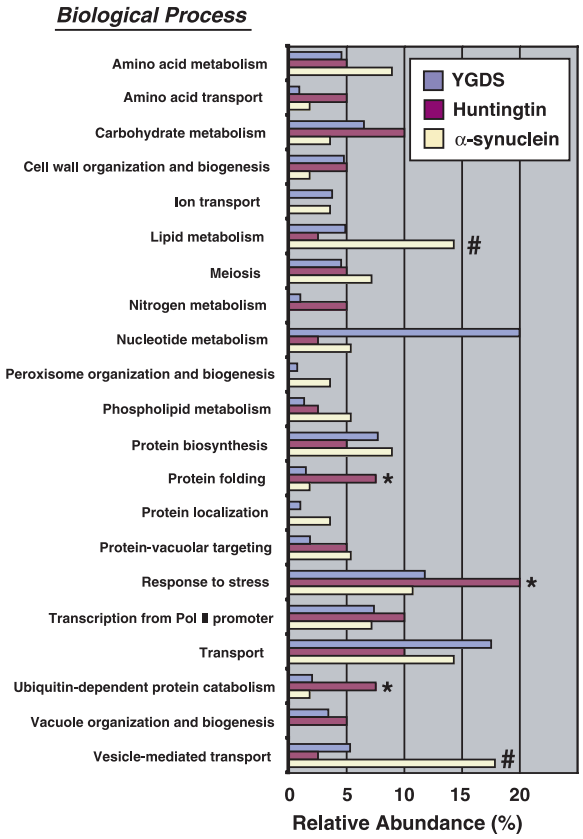

Fig. 2. Comparison of the relative percentage of genes in functional categories for the yeast gene deletion set (YGDS) and the huntingtin/α-synuclein synthetic lethal screens. Thirty-five percent (14/40) of genes that enhanced HD53Q toxicity clustered in the functionally related categories of response to stress, protein folding, and ubiquitin-dependent protein catabolism (*), whereas 32% (18/57) of genes that modified α-synuclein toxicity clustered in the functionally related categories of lipid metabolism and vesicle-mediated transport (#).

Table 1. Yeast strains synthetically sick or lethal with HD53Q. The ortholog category indicates yeast genes with human orthologs.

Strain	Ortholog	Function	Strain	Ortholog	Function
1. *apj1*Δ	Yes	Hsp40 chaperone	27. *rim4*Δ	Yes	RNA binding
2. *apm2*Δ	Yes	Nonselective vesicle transport	28. *sam2*Δ	Yes	S-adenosylmethionine synthetase 2
3. *ayr1*Δ	Yes	Ketoreductase; acylglycerone-phosphate reductase	29. *sas3*Δ	Yes	Histone acetyltransferase
4. *cit2*Δ	Yes	Citrate synthase, peroxisomal	30. *sdt1*Δ	No	5′-Nucleotidase
5. *cmk1*Δ	Yes	Protein histidine kinase	31. *sip18*Δ	No	Binds phospholipids
6. *cos111*Δ	No	Possibly involved in ubiquitin pathway	32. *sng1*Δ	No	Probable transport protein
7. *cps1*Δ	Yes	Gly-X carboxypeptidase	33. *stp2*Δ	Yes	Transcription factor
8. *dcg1*Δ	No	Possibly involved in cell wall biosynthesis	34. *tea1*Δ	No	Transcriptional activator
9. *fil1*Δ	No	Translation factor	35. *tvp15*Δ	No	Possibly involved in vesicular transport
10. *fpr2*Δ	Yes	Peptidyl-prolyl cis-trans isomerase	36. *ubp13*Δ	Yes	Ubiquitin C-terminal hydrolase
11. *gda1*Δ	Yes	Guanosine diphosphatase	37. *vps70*Δ	Yes	Possibly involved in vacuolar trafficking
12. *glo2*Δ	Yes	Hydroxyacylglutathione hydrolase	38. *yhb1*Δ	Yes	Nitric oxide dioxygenase; oxygen transporter
13. *gre2*Δ	Yes	Alpha-acetoxy ketone reductase	39. *yrb30*Δ	No	Unknown
14. *gsh2*Δ	Yes	Glutathione synthase	40. *ybr100w*Δ	No	Possibly involved in DNA damage repair
15. *hlj1*Δ	Yes	Hsp40 chaperone in ER	41. *ybr255w*Δ	No	Unknown
16. *hlr1*Δ	No	Unknown, similar to Lre1 (Pkc1p-MAPK pathway)	42. *ydr215c*Δ	No	Unknown
17. *hms1*Δ	Yes	Transcription factor	43. *ygr015c*Δ	Yes	Alpha or beta hydrolase fold family
18. *ipk1*Δ	No	Phosphatidylinositol phosphate kinase	44. *yjr107w*Δ	No	Has similarity to acylglycerol lipase
19. *kgd1*Δ	Yes	Alpha-ketoglutarate dehydrogenase	45. *ykr017c*Δ	Yes	Has a TRIAD composite zinc finger domain
20. *msb1*Δ	Yes	Activates Pkc1p-MAPK pathway	46. *ykr064w*Δ	No	Transcription factor
21. *mrp11*Δ	No	Protein of the mitochondrial large ribosomal subunit	47. *ylr128w*Δ	Yes	Basic helix-loop-helix leucine zipper protein
22. *mup1*Δ	Yes	Methionine permease	48. *ymr160w*Δ	No	Unknown
23. *pc16*Δ	No	Cyclin-dependent protein kinase	49. *ynl296w*Δ	No	Possibly involved in vacuolar trafficking
24. *phm8*Δ	No	Possibly involved in phosphate metabolism	50. *yor292c*Δ	Yes	Peroxisomal protein
25. *prm5*Δ	No	Possibly involved in cell stress	51. *yor300w*Δ	No	Bipolar budding and bud site selection
26. *psp1*Δ	No	Possibly involved in DNA replication	52. *ypl067c*Δ	No	Unknown

assays were conducted on protein lysates from 10 strains (fig. S2). No correlation between levels of aggregation and viability was observed in these strains (fig. S2).

Of the α-synuclein–sensitive mutants that were identified in the yeast screen, 66% (57/86) corresponded to genes for which a function or genetic role has been determined experimentally or can be predicted (Table 2). Thirty-two percent (18/57) of these genes clustered in the functionally related categories of lipid metabolism and vesicle-mediated transport (Fig. 2). As with the HD53Q screen, the remaining genes in the α-synuclein screen were distributed among numerous functional categories (Fig. 2), and a high percentage of the genes (50% or 43/86) have human orthologs (Table 2).

Evidence increasingly suggests that genes involved in response to stress, protein folding, and the ubiquitin-mediated protein catabolism play important roles in HD and the polyQ disorders (*16*, *17*). Overexpression of the chaperones Hsp40 and/or Hsp70 in fruit fly and mouse models of polyQ disorders suppresses neurodegeneration, whereas mutation of genes involved in ubiquitin-mediated protein catabolism enhances neurodegeneration (*18–22*). Our yeast screen identified two Hsp40 homologs (Apj1 and Hlj1) that when deleted enhance toxicity of HD53Q. These results indicate that in wild-type yeast cells Hsp40 chaperones are necessary to suppress polyQ toxicity. In addition to chaperones, eight genes (*FPR2*, *GRE2*, *GSH2*, *HLR1*, *PRM5*, *SIP18*, *YHB1*, and *YJR107W*) involved in various forms of cellular stress (osmotic, oxidative, and nitrosative) and three genes involved in ubiquitin-mediated protein catabolism (*UBP13*, *YBR203W*, and *YKR017C*) were identified as enhancers of HD53Q toxicity in yeast.

Several genes in the functional categories of response to stress and ubiquitin-mediated protein catabolism were also isolated in the α-synuclein screen (*SOD2*, *GTT1*, *HSP30*, *TSL1*, and *UBC8*) (Table 2). However, in contrast to the HD53Q screen, the levels of genes in these categories were not increased above background levels (Fig. 2). A recent study demonstrated that Hsp70 overexpression rescues α-synuclein–induced neurodegeneration in a *Drosophila* model for PD (*23*). Although considerable genetic, cell biological, and biochemical evidence suggests that genes involved in response to stress, protein folding, and ubiquitin-mediated protein catabolism play important roles in the pathobiology of PD, the results from the α-synuclein yeast screen indicate that genes involved in lipid metabolism and vesicle-mediated transport may also be primary pathways that regulate toxicity of α-synuclein.

α-Synuclein localizes to nerve terminals and may be associated with synaptic vesicles, based on immunohistochemistry and ultra-

Table 2. Yeast strains synthetically sick or lethal with α-synuclein. The ortholog category indicates yeast genes with human orthologs.

Strain	Ortholog	Function	Strain	Ortholog	Function
1. *ape2*Δ	Yes	Aminopeptidase	44. *tsl1*Δ	No	Alpha,alpha-trehalose-phosphate synthase
2. *arl3*Δ	Yes	ARF small monomeric GTPase activity	45. *ubc8*Δ	Yes	Ubiquitin-conjugating enzyme
3. *aro1*Δ	No	Arom pentafunctional enzyme	46. *vps24*Δ	Yes	Sorts proteins in the prevacuolar endosome
4. *cog6*Δ	Yes	Involved in vesicular transport to the Golgi	47. *vps28*Δ	Yes	Required for traffic to vacuole
5. *crh1*Δ	Yes	Cell wall protein	48. *vps60*Δ	No	Vacuolar protein sorting
6. *cvt17*Δ	No	Lipase	49. *war1*Δ	No	Transcription factor
7. *dpp1*Δ	Yes	Diacylglycerol pyrophosphate phosphatase	50. *yat1*Δ	Yes	Outer carnitine acetyltransferase, mitochondrial
8. *fun26*Δ	Yes	Nucleoside transporter	51. *ybr013c*Δ	No	Unknown
9. *gip2*Δ	Yes	Regulatory subunit for PP1 phosphatase	52. *ybr284w*Δ	Yes	AMP deaminase
10. *glo4*Δ	Yes	Hydroxyacylglutathione hydrolase	53. *ybr300c*Δ	No	Unknown
11. *gtt1*Δ	No	Glutathione transferase	54. *ycl042w*Δ	No	Unknown
12. *hbs1*Δ	Yes	~To translation elongation factor EF-1alpha	55. *ycr026c*Δ	Yes	Contains type I phosphodiesterase domain
13. *hsp30*Δ	No	Heat shock protein for pH homeostasis	56. *ycr050c*Δ	No	Unknown
14. *ino4*Δ	No	Transcript. factor (phospholipid syn. genes)	57. *ycr051w*Δ	Yes	Contains ankyrin (Ank) repeats
15. *mad1*Δ	No	Involved in spindle-assembly checkpoint	58. *ycr085w*Δ	No	Unknown
16. *mal31*Δ	Yes	Maltose transporter	59. *ydl118w*Δ	No	Possibly involved in meiotic nuclear division
17. *mei4*Δ	No	Required for meiotic recombination	60. *ydr154c*Δ	No	Unknown
18. *met17*Δ	Yes	O-acetylhomoserine (thiol)-lyase	61. *ydr220c*Δ	No	Unknown
19. *met32*Δ	Yes	Transcription factor	62. *yfr035c*Δ	No	Unknown
20. *msb3*Δ	Yes	RAB GTPase activator	63. *ygl109w*Δ	No	Unknown
21. *nbp2*Δ	Yes	Poss. involved in cytoskeletal organization	64. *ygl165c*Δ	No	Unknown
22. *nit2*Δ	Yes	Nitrilase	65. *ygl226w*Δ	No	Unknown
23. *nup53*Δ	Yes	Component of nuclear pore complex	66. *ygl231c*Δ	Yes	Unknown
24. *opi3*Δ	Yes	Phosphatidylethanolamine N-methyltransf.	67. *ygl262w*Δ	No	Unknown
25. *pca1*Δ	Yes	P-type copper-transporting ATPase	68. *ygr130c*Δ	Yes	Unknown
26. *pex2*Δ	Yes	Peroxisomal biogenesis protein	69. *ygr154c*Δ	No	Unknown
27. *pex8*Δ	No	Peroxisomal biogenesis protein	70. *ygr201c*Δ	Yes	Translation elongation factor
28. *pho13*Δ	Yes	4-Nitrophenylphosphatase	71. *ygr290w*Δ	No	Unknown
29. *pox1*Δ	Yes	Acyl-CoA oxidase	72. *yhr199c*Δ	No	Unknown
30. *ptk2*Δ	Yes	Serine/threonine protein kinase	73. *yjl118w*Δ	No	Unknown
31. *rpl41a*Δ	No	Structural constituent of ribosome	74. *yjl122w*Δ	No	Unknown
32. *rny1*Δ	Yes	Endoribonuclease	75. *yjl135w*Δ	No	Unknown
33. *sac2*Δ	Yes	Involved in protein sorting in the late Golgi	76. *yjr154w*Δ	No	Unknown
34. *sap4*Δ	No	Serine/threonine phosphatase	77. *ykl098w*Δ	No	Unknown
35. *sod2*Δ	Yes	Manganese superoxide dismutase	78. *ykl100c*Δ	Yes	Unknown
36. *stf1*Δ	No	ATPase inhibitor	79. *ykr023w*Δ	Yes	Unknown
37. *stp2*Δ	Yes	Transcription factor	80. *ykr035c*Δ	No	Unknown
38. *suv3*Δ	Yes	Mitochondrial RNA helicase (DEAD box)	81. *ylr365w*Δ	No	Unknown
39. *swr1*Δ	Yes	Member of Snf2p DNA helicase family	82. *ylr376c*Δ	No	Possibly involved in DNA repair
40. *thi7*Δ	No	Thiamin transporter	83. *ymr226c*Δ	Yes	Oxidoreductase
41. *tlg2*Δ	Yes	Syntaxin homolog (t-SNARE)	84. *yml089c*Δ	No	Unknown
42. *thr1*Δ	No	Homoserine kinase	85. *ymr289w*Δ	No	Unknown
43. *tna1*Δ	Yes	Nicotinamide mononucleotide permease	86. *ypl136w*Δ	No	Unknown

structural analyses (*24*). α-Synuclein binds lipid membranes (*25–27*) and can inhibit phospholipase D2 in vitro (*28*). α-Synuclein interacts with synphilin-1 (*29*), which has been proposed to function as an adaptor protein linking α-synuclein to proteins involved in vesicular transport. Although the precise function of α-synuclein is still not clear, this protein has been linked to learning, development, and plasticity (*30*) and most likely plays a role in synaptic vesicle recycling. Recent in vitro studies suggest that prefibrillar intermediates called protofibrils formed by α-synuclein can bind and permeabilize acidic phospholipid vesicles (*31*), which has been proposed to lead to defective sequestration of dopamine into vesicles and subsequent generation of reactive oxygen species in the cytoplasm that contribute to neuronal dysfunction and cell death (*32*). Taken together, these results are consistent with those from our α-synuclein genetic screen and with studies examining the biological and pathobiological effects of α-synuclein in yeast. α-Synuclein, and not a mutant huntingtin fragment, localized to membranes, caused the accumulation of lipid droplets and inhibited phospholipase D and vesicular trafficking (*11*). The results from the yeast screen are also consistent with recent expression-profiling studies in *Drosophila* that overexpress α-synuclein, which showed that lipid and membrane transport mRNAs were tightly associated with α-synuclein expression (*33*).

The results from yeast screens clearly indicate that toxicity mediated by α-synuclein and a mutant huntingtin fragment is regulated by nonoverlapping sets of conserved genes and pathways. The major functional categories enriched in the α-synuclein genetic screen did not overlap with any of the major categories observed in the HD53Q screen, and only 1 out of 138 genes that enhanced toxicity was found in common to both screens (*STP2*). Collectively, these results suggest that distinct pathogenic mechanisms may underlie HD and PD.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/302/5651/1769/DC1
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Figs. S1 and S2
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Yeast Cells Provide Insight into Alpha-Synuclein Biology and Pathobiology

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Alpha-synuclein is implicated in several neurodegenerative disorders, such as Parkinson's disease and multiple system atrophy, yet its functions remain obscure. When expressed in yeast, alpha-synuclein associated with the plasma membrane in a highly selective manner, before forming cytoplasmic inclusions through a concentration-dependent, nucleated process. Alpha-synuclein inhibited phospholipase D, induced lipid droplet accumulation, and affected vesicle trafficking. This readily manipulable system provides an opportunity to dissect the molecular pathways underlying normal alpha-synuclein biology and the pathogenic consequences of its misfolding.

Alpha-synuclein (αSyn) is abundant and broadly expressed in the brain, where it interacts with membranes, vesicular structures, and a puzzling variety of other proteins (*1*). Some cases of Parkinson's disease (PD) have a genetic basis (*2*) that implicates protein folding and quality-control (QC) factors, including a ubiquitin ligase (*3*) and a ubiquitin C-terminal hydrolase (*4*, *5*), in αSyn pathology. In mammalian cells αSyn has been reported in the nucleus, cytosol, associated with membranes and, in diseased brains, in large cytoplasmic inclusions (Lewy bodies) (*1*). Synucleinopathies are now classified as protein-misfolding disorders (*6*). Given the strong conservation of protein folding, membrane trafficking, and protein QC mechanisms between yeast and higher eukaryotes, we used *Saccharomyces cerevisiae* to uncover and establish basic aspects of both normal and abnormal αSyn biology.

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To study αSyn dynamics in living cells, we created an αSyn-GFP (green fluorescent protein) fusion that was not subject to proteolysis in yeast cells (Fig. 1A) (*7*) as related fusions have been in mammalian cells (*8*). Integrating this construct into the genome under the control of a galactose-inducible promoter allowed routine manipulations in the absence of αSyn expression. Upon induction with galactose, wild-type (WT) αSyn-GFP localized intensely at the plasma membrane; a smaller quantity accumulated in the cytoplasm (Fig. 1B). Compared with other GFP fusion proteins, αSyn did not localize to mitochondrial or nuclear membranes (*9*). Thus, reminiscent of its selectivity for membranes with particular lipid compositions in vitro (*10*), αSyn has a high intrinsic selectivity for particular cellular membranes in vivo.

Two αSyn point mutants (A53T and A30P) are associated with rare forms of early-onset familial PD but have distinct physical properties (*11*, *12*). In yeast, each αSyn mutant accumulated at the same level as WT αSyn (Fig. 1A, one copy), but their cellular distributions