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REVIEW ARTICLE

Molecular Genetics Approaches in Yeast to Study Amyloid Diseases

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Abstract

The occurrence of protein aggregates in ordered fibrillar structures known as amyloid, found inside and outside of brain cells, is a feature shared by many neurodegenerative disorders, including Alzheimer's, Parkinson's, and Huntington's diseases. Although the molecular mechanisms that underlie neurodegeneration will ultimately have to be tested in neuronal and animal models, there are several distinct advantages in using model organisms to elucidate fundamental aspects of protein aggregation, amyloid formation, and toxicity. Here, we review recent studies indicating that amyloid formation by disease-causing proteins can be faithfully recapitulated in simple yeast-based models in *Saccharomyces cerevisiae*. These studies have already contributed to our basic understanding of molecular chaperone function/dysfunction in Huntington's disease, and functional genomics approaches being undertaken currently will likely bear novel insights into the genes and pathways that modulate neuronal cell dysfunction and death in these devastating diseases. A final advantage of using yeast to study amyloid formation and toxicity is the ease and rapidity with which large-scale drug-screening efforts can be conducted in this model organism.

Index Entries: Neurodegeneration; amyloid; yeast genetics; disease modifiers; chemical genetics.

The Awesome Power of Yeast Genetics

The baker's yeast *Saccharomyces cerevisiae*, which has been used as a biotechnological tool for many centuries, is a commonly used model organism because basic cellular mechanisms, such as replication, recombination, cell division, protein folding, intracellular transport, and metabolism are well-conserved between yeast and higher eukaryotes, including mammals. Properties that make yeast an incredibly useful model for molecular genetics approaches include rapid growth on defined media, dispersed cells, the ease of replica plating and mutant isolation, a well-defined genetic system, and most

important, a highly versatile DNA transformation system (Sherman, 1991). Because yeast can exist in a stable haploid or diploid state, it is an ideal organism on which to perform classical genetics. Its genome of 14 Mb was the first eukaryotic genome to be fully sequenced, which has enabled more extensive annotation than other sequenced genomes (Goffeau et al., 1996). The single greatest advantage of yeast as a model system lies in the ability to rapidly perform genetic manipulations and screen for induced phenotypes. Any single gene can be knocked out or over-expressed with experiments that take a matter of days. In addition, the yeast two-hybrid technique provides a genetic means to identify proteins that

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physically interact *in vivo*. Because of the “awesome power of yeast genetics,” a growing number of molecular biologists use yeast as a primary research tool.

Approaches to Modeling Protein Aggregation, Amyloid Formation, and Toxicity in Yeast

Although yeast cells cannot be used to study neurodegeneration directly *per se*, numerous studies to date suggest that cell autonomous biological response pathways exist that are relevant to amyloid diseases, and these pathways are beginning to be characterized in yeast and in other model organisms. The utility of yeast as a model system for studies relating to basic mechanisms of protein misfolding, aggregation, and toxicity has already resulted in significant insights into pathogenesis of Huntington’s disease (HD; reviewed below). It is important to note that the yeast two-hybrid technique has also been used extensively in studies relating to neurodegenerative disorders; however, we will not review this literature here. In this article, we will discuss genetic and biochemical studies in which yeast has been used as a test tube to study proteins that cause Friedreich’s ataxia (FRDA), amyotrophic lateral sclerosis (ALS), HD, and Parkinson’s disease (PD) (Table 1). We will also describe functional genomics approaches in yeast to study amyloid diseases. The power of yeast models for investigating how conformational changes in proteins affect cell function is illustrated by studies of prion-like factors in yeast, which have already yielded major insights into basic mechanisms of prion biology (Lindquist et al., 2001). These studies are reviewed by Thomas Scheibel in this issue (Amyloid Formation of a Yeast Prion Determinant) and will not be covered here.

FRDA

One of the first neurodegenerative diseases to be modeled in yeast is FRDA, an autosomal recessive disease characterized by progressive gait and limb ataxia, signs of axonal sensory neuropathy, pyramidal weakness of the legs, and dysarthria (Puccio and Koenig, 2000). The vast majority of patients with FRDA have a GAA trinucleotide repeat expansion in the gene *frataxin*, identified by positional cloning as the gene mutated in this disease (Campuzano et al., 1996). The expansion causes reduced expression of the protein. The *frataxin* sequence is similar

to a yeast gene of previously unknown function, subsequently named *YFH1* (yeast *frataxin* homolog) (Babcock et al., 1997). Yeast strains lacking *YFH1* are unable to grow on nonfermentable carbon sources, indicating defective oxidative phosphorylation because of impaired mitochondrial function (Babcock et al., 1997; Foury and Cazzalini, 1997; Koutnikova et al., 1997; Wilson and Roof, 1997). In addition, iron accumulates in mitochondria to high levels in *yfh1* mutants, suggesting that iron accumulation and oxidative damage might play an important role in the etiology of FRDA. Both frataxin and Yfh1p localize to mitochondria. Importantly, wild-type human *frataxin* complements strains lacking *YFH1*, whereas a point mutant allele from a patient with FRDA only partially complements the yeast mutant (Cavadini et al., 2000). Friedreich’s ataxia (FRDA) is one of the first neurodegenerative disorders in which a pathogenic mechanism was elucidated directly from studies in yeast, and a more detailed description of these studies can be found elsewhere (Knight et al., 1999).

ALS

Amyotrophic lateral sclerosis (ALS) is a devastating neurological disorder that rapidly progresses from mild motor symptoms to severe paralysis and premature death, resulting from the selective death of motor neurons. A significant percentage of patients with familial ALS carry a mutation of *SOD1* (the gene coding for the Cu,Zn superoxide dismutase), an enzyme that catalyzes the disproportion of superoxide radicals to dioxygen and hydrogen peroxide. Interestingly, >90 mutations in *SOD1* have been described thus far, and all of them cause familial ALS, with one exception. SOD constitutes an important defense mechanism against oxidative stress in organisms ranging from microbes to plants and animals (Fridovich, 1995). As with *frataxin*, the human *SOD1* gene can complement yeast *sod1* mutants (Nishida et al., 1994; Rabizadeh et al., 1995; Corson et al., 1998, 1999). Studies in yeast and mice indicate that ALS is not caused by reduced SOD activity but, rather, by a dominant gain of function of mutant Sod1 protein (Bruijn et al., 1998; Cleveland, 1999; Cleveland and Rothstein, 2001). Although the molecular basis of this dominant gain of function is poorly understood, studies to elucidate the molecular mechanisms that underlie ALS are actively being pursued in yeast, neuronal, and animal models.

Table 1
Human Neurological Disorders Modeled in Yeast

Disease	Main protein involved	Yeast ortholog	Presence of inclusions in disease	References
AD	APP	No	Yes	Greenfield et al. (1999); Zhang et al. (1997)
PD	α -Synuclein	No	Yes	Outeiro and Lindquist (2003)
PolyQ expansion diseases (e.g., HD)	Several (e.g., Huntingtin)	No	Yes	Krobitsch and Lindquist (2000); Meriin et al. (2002); Muchowski et al. (2000)
Prion encephalopathies	PrP	No, but yeast has prions	Yes	Uptain and Lindquist (2002)
ALS	SOD-1	Yes	Yes	Nishida et al. (1994)
FRDA	Frataxin	Yes	No	Knight et al. (1999)

HD

Huntington's disease (HD) is a devastating neurodegenerative disorder that affects at least 1 in 10,000 people in the United States. The mutation that causes HD is an expansion of CAG repeats (encoding polyglutamine [polyQ]) in the gene *IT-15* (The Huntington's Disease Collaborative Research Group, 1993). The CAG repeat number is polymorphic in the general population, varying between 4 and 35. Individuals affected by HD have >35 repeats. The mutation is inherited in an autosomal-dominant manner, and the same mutational mechanism is responsible for a growing number of less common neurodegenerative disorders, including the spinocerebellar ataxias. In HD, cells in the striatum and cortex are most affected, resulting in progressive chorea, rigidity, and dementia. The major neuropathological hallmark in HD is the presence of brain lesions composed of intranuclear and cytoplasmic inclusions that contain the protein huntingtin (encoded by *IT-15*). The length of the polyQ expansion in huntingtin correlates directly with kinetics of its aggregation in vitro, and indirectly with age of onset and severity of the disease in HD patients.

Several research groups recently have reported models in yeast to study the folding and behavior of proteins with expanded polyQ tracts (Krobitsch and Lindquist, 2000; Muchowski et al., 2000; Meriin et al., 2002). Heterologous expression of the first exon of huntingtin in yeast as a fusion to the green

fluorescent protein (GFP) results in a polyQ length-dependent aggregation and formation of cytoplasmic inclusions that can easily be detected in living cells by fluorescence microscopy (Fig. 1A). A simple filter-retention assay can also be used to isolate detergent-insoluble aggregates formed by polyQ proteins with expanded repeats in the disease-causing range (Fig. 1B). Although yeast models were developed ultimately to perform large-scale genetic or chemical genetic approaches to understand the basic mechanisms of protein aggregation and toxicity (*see below*), studies to date have used a reductionist approach, that is, studying the effects of one gene family or cellular pathway at a time. This approach has been fruitful, as evidenced by studies showing that overexpression of molecular chaperones profoundly alters the aggregation state of polyQ proteins in vivo (Krobitsch and Lindquist, 2000; Muchowski et al., 2000). Overexpression of heat shock proteins Hsp70 or Hsp40 inhibited the formation of large, detergent-insoluble inclusions, resulting instead in the accumulation of detergent-soluble inclusions. Overexpression of Hsp104, a yeast-specific disaggregase, also exhibited similar properties. Since 1998, 15 publications have reported the effects of overexpression of chaperones in cellular models of polyQ aggregation and toxicity. Despite the various polyQ proteins and cell types used in these studies, in general these papers reported similar results, showing that members of the Hsp70 and Hsp40 families of chaperones suppress the

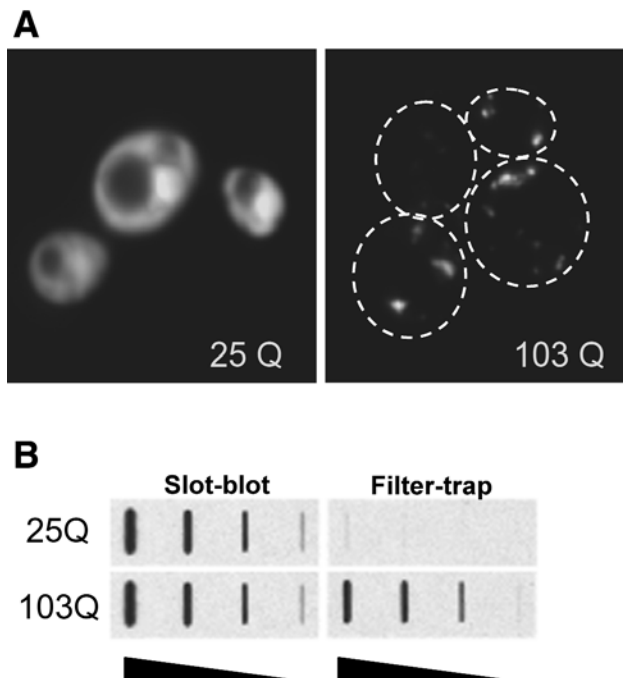


Fig. 1. Aggregation and inclusion body formation in a yeast model of polyQ aggregation. **(A)** Fluorescence microscopy of yeast cells expressing huntingtin exon 1 with either 25 or 103 glutamines fused to GFP. The presence of cytoplasmic inclusions is observed with 103Q but not with 25Q. **(B)** Slot-blot and filter-trap analysis of polyQ aggregates in yeast. Ten micrograms of a total yeast cell lysate (after glass bead lysis) was serially diluted (fivefold dilutions), boiled in SDS, filtered through a nitrocellulose (slot-blot) or cellulose-acetate (filter-trap) membrane (0.2- μ m pores), washed, and probed with an anti-huntingtin antibody. Large (>0.2- μ m), detergent-insoluble polyQ aggregates from yeast cells expressing 103Q are retained in the filter trap, whereas the 25Q protein remains soluble and flows through the membrane.

aggregation and toxicity of polyQ-containing proteins (Sherman and Goldberg, 2001). More importantly, several exciting studies have shown recently that chaperones can also protect against neurodegeneration in animal models of polyQ disease and PD (Muchowski, 2002). The molecular mechanisms by which chaperones protect against disease in animal models remain poorly understood.

We recently reported experiments that addressed mechanisms of aggregation and inclusion body formation by mutant huntingtin fragments in yeast and in mammalian cells (Muchowski et al., 2002). Treatment of yeast cells with drugs that disrupt microtubules resulted in <2% of the detergent-insoluble polyQ protein observed in mock-treated cells and prevented the formation of large, juxtanuclear inclusions. Disruption of the microtubule cytoskeleton unmasked a potent glutamine length-dependent toxicity of polyQ proteins in which the polyQ proteins exist in an entirely detergent-soluble, nonaggregated state. Results from the yeast model paralleled those from neuronal pheochromocytoma cells, in which

disruption of microtubules eliminated the formation of juxtanuclear and intranuclear inclusions by polyQ proteins. Our results suggest that active transport along microtubules might be required for inclusion formation by polyQ proteins, and that inclusion formation might have evolved as a cellular mechanism to promote the sequestration or clearance of soluble species of polyQ proteins that might otherwise be toxic to cells (Muchowski et al., 2002). The microtubule-dependent formation of inclusions by polyQ proteins recently has been confirmed (Shimohata et al., 2002). It is now apparent that polyQ proteins share many, if not all, of the properties of an aggresome (Bence et al., 2001; Waelter et al., 2001), a pericentriolar structure composed of an accumulation of misfolded protein originally described for the cystic fibrosis transmembrane conductance regulator (Johnston et al., 1998). Aggresomes are now known to be formed by numerous and diverse proteins, and might represent a general cellular response to the presence of an excess of misfolded protein (Kopito, 2000).

Although significant efforts have been made to understand the role of huntingtin and the molecular mechanisms that underlie neurodegeneration in HD, a unifying pathogenic mechanism has not been resolved. Published data exist to support the following mechanisms that are not necessarily mutually exclusive: (1) aggregation of huntingtin into a toxic oligomeric/protofibrillar/fibrillar species that perturbs the structure and/or function of downstream targets; (2) activation of caspases or other proteases that mediate the cleavage of huntingtin into a toxic species; (3) impairment of the ubiquitin-proteasome system; (4) impairment of molecular chaperone function; (5) localization of huntingtin in the cell nucleus and inhibition of gene transcription; (6) impairment of mitochondrial function and/or oxidative stress; (7) impairment of intracellular trafficking pathways and synaptic transmission; and (8) excitotoxicity (Tobin and Signer, 2000; Zoghbi and Orr, 2000; Ross, 2002). If HD does indeed involve multiple disease mechanisms, it will be crucial to understand in a temporal sense the genes and pathways that are of primary significance for disease initiation, progression, and ultimately death. In principle, genetic approaches in yeast could be used to delineate such pathways (*see below*).

PD

Parkinson's disease (PD) is a progressive, neurodegenerative disorder that affects about 2% of people over 65 yr old and 4–5% of people over 85 (between 1 and 1.5 million Americans). PD is characterized by loss of dopaminergic neurons in the substantia nigra and is accompanied by muscle rigidity, bradykinesia, resting tremor, and postural instability. The neuropathological hallmark of idiopathic PD is the presence of brain lesions (Lewy bodies) that are composed primarily of the protein α -synuclein (Goedert, 2001). Two point mutations in the α -synuclein gene, resulting in A30P or A53T substitutions in the protein, cause early-onset, inherited forms of PD. Recently, Singleton et al. reported a triplication of the α -synuclein locus in a family with PD, suggesting the levels of the protein play an important role in the disease. α -Synuclein exists in Lewy bodies as an ordered fibrillar structure generically termed amyloid. Proteins that cause or are associated with HD, Alzheimer's disease (AD), and other neurodegenerative diseases also aggregate into amyloid structures. The amyloid hypothesis (developed originally for AD) states that the aggregation of proteins into an ordered fibrillar

structure is causally related to aberrant protein interactions that culminate in neuronal dysfunction and ultimately neurodegeneration (Hardy and Selkoe, 2002).

α -Synuclein assembles into fibrils that also share many, if not all, of the characteristics displayed by amyloid fibrils (Conway et al., 2000a). α -Synuclein is a natively unfolded protein with little ordered secondary structure, which polymerizes into ~10-nm fibrils *in vitro*. These fibrils display biochemical and ultrastructural features that are present in PD brain tissue (Rochet and Lansbury, 2000). α -Synuclein mutations that cause inherited forms of PD (A30P and A53T) accelerate the oligomerization of α -synuclein into structured fibrillization intermediates, called protofibrils, which are hypothesized to be responsible for neuronal death in PD (Conway et al., 2000b). Recent studies in transgenic mice and fruit flies are consistent with an important role for α -synuclein-dependent toxicity in PD. In these models, overexpression of α -synuclein or its mutants leads to progressive neuropathological and motor abnormalities (Feany and Bender, 2000; Kahle et al., 2000; Masliah et al., 2000; van der Putten et al., 2000; Giasson et al., 2002).

α -Synuclein is an abundant brain protein that is in a family with at least three members (α , β and γ -synuclein); however, the functions and structures of these proteins are not well-understood (Goedert, 2001). α - and β -Synuclein localize to nerve terminals and might be associated with synaptic vesicles, as judged by immunohistochemistry and ultrastructural analyses (Clayton and George, 1999). Targeted disruption of α -synuclein in mice does not result in Lewy body formation and is not associated with neurodegeneration (Abeliovich et al., 2000). However, as in patients with PD, the brains of mice lacking α -synuclein are characterized by reduced levels of dopamine. α -Synuclein binds lipid membranes *in vitro* (Davidson et al., 1998; Jensen et al., 1998; Perrin et al., 2000; Sharon et al., 2001) and can inhibit phospholipase D (Jenco et al., 1998; Ahn et al., 2002). The yeast two-hybrid technique identified synphilin-1 as a protein that interacts with α -synuclein (Engelender et al., 1999). Although the function of synphilin-1 is also unknown, it has been proposed to function as an adaptor protein linking α -synuclein to proteins involved in vesicular transport. Although the function of α -synuclein is still not clear, it has been linked to learning, development, and plasticity (George et al., 1995) and most likely plays a role in synaptic vesicle recycling. Interest-

ingly, recent *in vitro* studies suggest that α -synuclein protofibrils can bind and permeabilize acidic phospholipid vesicles (Volles et al., 2001; Volles and Lansbury, 2002). It has been proposed that this might lead to defective sequestration of dopamine into vesicles and subsequent generation of reactive oxygen species in the cytoplasm, which contribute to neuronal dysfunction and cell death (Lotharius and Brundin, 2002).

In spite of the tremendous effort put into generating and characterizing animal models for PD, the molecular mechanisms that underlie neurodegeneration in PD are not well defined. It is therefore important to continue to develop simple model systems amenable to genetic manipulation that might provide important leads for the development of more significant animal models and, ultimately, to novel neuroprotective agents for the treatment of PD and related synucleinopathies. Toward this goal, we have recently developed a yeast-based model to study the folding, behavior, and basic biological properties of α -synuclein and its disease-causing mutants (Outeiro and Lindquist, 2003). In this model, yeast cells express wild-type (WT) human α -synuclein, or the disease-causing mutants A30P and A53T, either alone or fused to the GFP under control of the *GAL1* promoter. Analysis of yeast cells by fluorescence microscopy shows that α -synuclein (WT) and A53T form several inclusion bodies in the yeast cytoplasm, whereas the fluorescence pattern for the A30P mutant is exclusively cytoplasmic (Outeiro and Lindquist, 2003). These results are consistent with immunolocalization studies of WT and mutant α -synuclein in primary cortical neurons (McLean et al., 2001). The yeast model has been used to perform large-scale genetic and chemical genetic approaches to understanding basic mechanisms of protein aggregation and toxicity (Willingham et al., 2003), and is also being used to screen for small molecules that can inhibit α -synuclein aggregation and/or toxicity (see below).

Functional Genomics Approaches to Studying Protein Aggregation, Amyloid Formation, and Toxicity in Yeast

The *S. cerevisiae* genome was the first to be sequenced from a eukaryotic organism 7 yr ago (Goffeau et al., 1996). The availability of well-annotated sequence information and the ease with which genetic manipulation can be performed in yeast have quickly led to a number of pioneering

studies that utilize genomics and proteomics approaches. For example, the yeast two-hybrid technique has been used successfully to define >2700 putative interactions involving at least 2000 different proteins (Schwikowski et al., 2000; Uetz et al., 2000). In a separate study, the subcellular localizations for over 1300 proteins tagged with GFP were determined (Ross-Macdonald et al., 1999). The entire set of predicted yeast proteins has also been fused to glutathione S-transferase (GST). The GST fusion proteins can be purified as 64 pools of 96 proteins each, and these pools have been mined for novel biochemical activities (Martzen et al., 1999).

Our studies take advantage of a collection of gene deletion mutants of *S. cerevisiae*, originally developed by the Saccharomyces Genome Deletion Project (Winzeler et al., 1999; Giaever et al., 2002). This collection contains 4850 viable mutant haploid strains, each lacking a single gene. The collection of yeast gene deletion strains (YGDS) has been used successfully to identify new genes and pathways involved in tolerance to radiation (Birrell et al., 2001), as well as human mitochondrial disease (Steinmetz et al., 2002), and to characterize the effects of pharmacological agents (Chan et al., 2000). We have used the YGDS to test the hypothesis that the downstream targets and molecular mechanisms by which huntingtin and α -synuclein mediate toxicity are unique (Willingham et al., 2003). The YGDS (4850 yeast strains, each lacking a single gene) was transformed with constructs that express huntingtin or α -synuclein. From these strains we isolated 52 genes that are synthetically sick or lethal with huntingtin and 86 genes that are synthetically sick or lethal with α -synuclein. Thirty percent of genes that affect huntingtin toxicity are found in the functionally related categories of protein folding and cell stress, whereas 29% of genes that modify α -synuclein toxicity are involved in vesicular transport and lipid metabolism. Our preliminary results indicate, surprisingly, that the genes and cellular pathways that modulate huntingtin and α -synuclein toxicity in yeast are completely divergent. Nearly half of the genes that we isolated are annotated as having one or more human ortholog, suggesting that we might have discovered in yeast conserved cell-biological response pathways to huntingtin and α -synuclein that are relevant to HD and PD. It should be mentioned that although previous genetic screens in *Drosophila* identified modifiers of polyQ toxicity (Fernandez-Funez et al., 2000; Kazemi-Esfarjani and Benzer, 2000), these

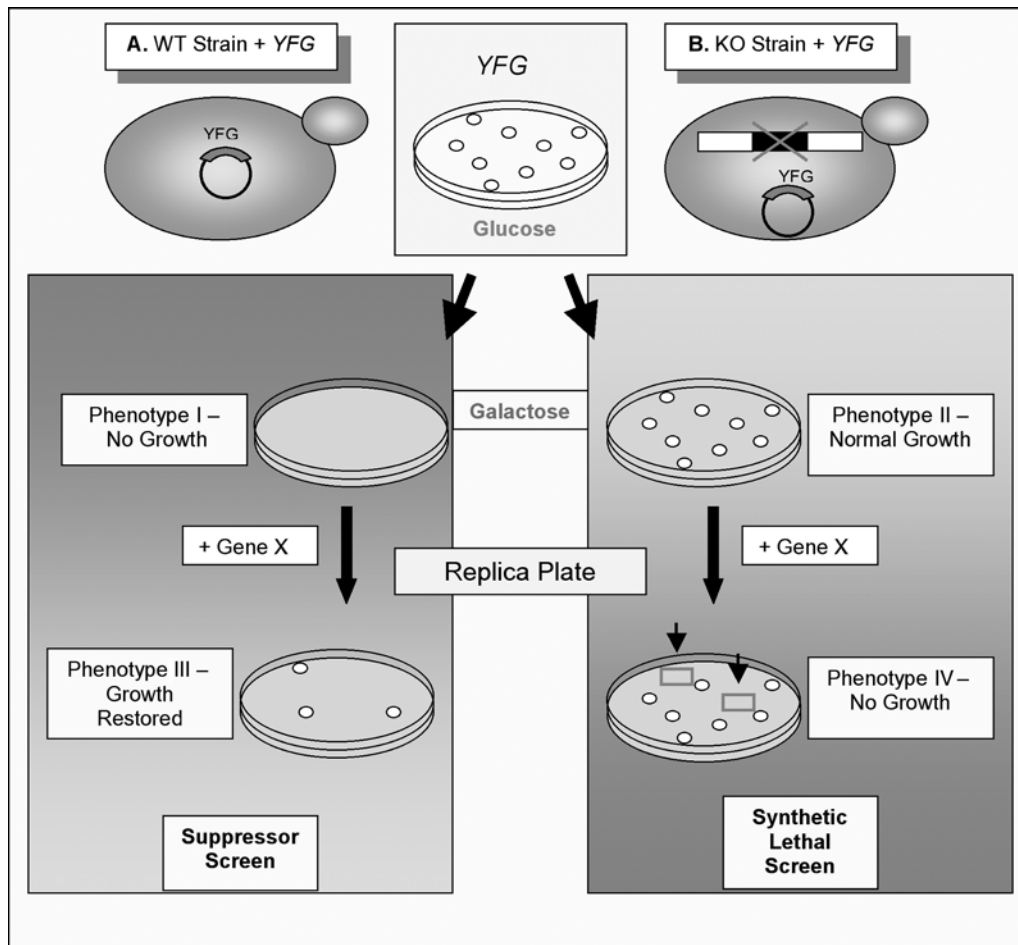


Fig. 2. An example of two simple genetic screens that can be performed using yeast as a model system. Yeast cells, either WT or bearing a knockout (KO) gene, are transformed with plasmids driving the expression of your favorite gene (YFG) from the GAL-inducible promoter, and plated onto selective media containing glucose (expression is repressed). Upon transfer to medium containing galactose, expression of YFG is induced, resulting either in no cell growth (phenotype I) or in normal cell growth (phenotype II). By introducing a second genetic element into the cells, the initial phenotypes can give rise to a situation where growth is restored (phenotype III) or there is loss of growth (phenotype IV). These types of genetic screens are called suppressor screens or synthetic lethal screens, respectively.

screens have inherent biases, and genes contributing to a phenotype might have been missed. Thus, a major advantage to studies with the YGDS is the knowledge that a large percentage of genes in the yeast genome (78% or 4850/6223) can be tested for their ability to modulate toxicity.

In principle, this type of screen could be used to identify genetic modifiers (suppressors or enhancers) for any neurodegenerative disease in which a gene product has been linked directly to pathogenesis (Fig. 2). In a typical screen, a function or genetic role for the majority of identified genes that modify toxicity has already been determined experimentally or can be predicted (Saccharomyces

Genome Database; <http://genome-www.stanford.edu/Saccharomyces/>). Using these annotations, genetic modifiers can be grouped into major functional categories (<https://www.incyte.com/proteome/YPDsearch-long.html>). A typical analysis would be to compare the relative percentages of genes in functional categories obtained in a screen with the percentage of genes in each category across the entire set of genes in the YGDS (Fig. 3).

It should be acknowledged that yeast as a model system for studies relating to neurodegeneration has its obvious limitations, with a major caveat being simply that some genes important for modulating neurodegeneration might not be present in the yeast

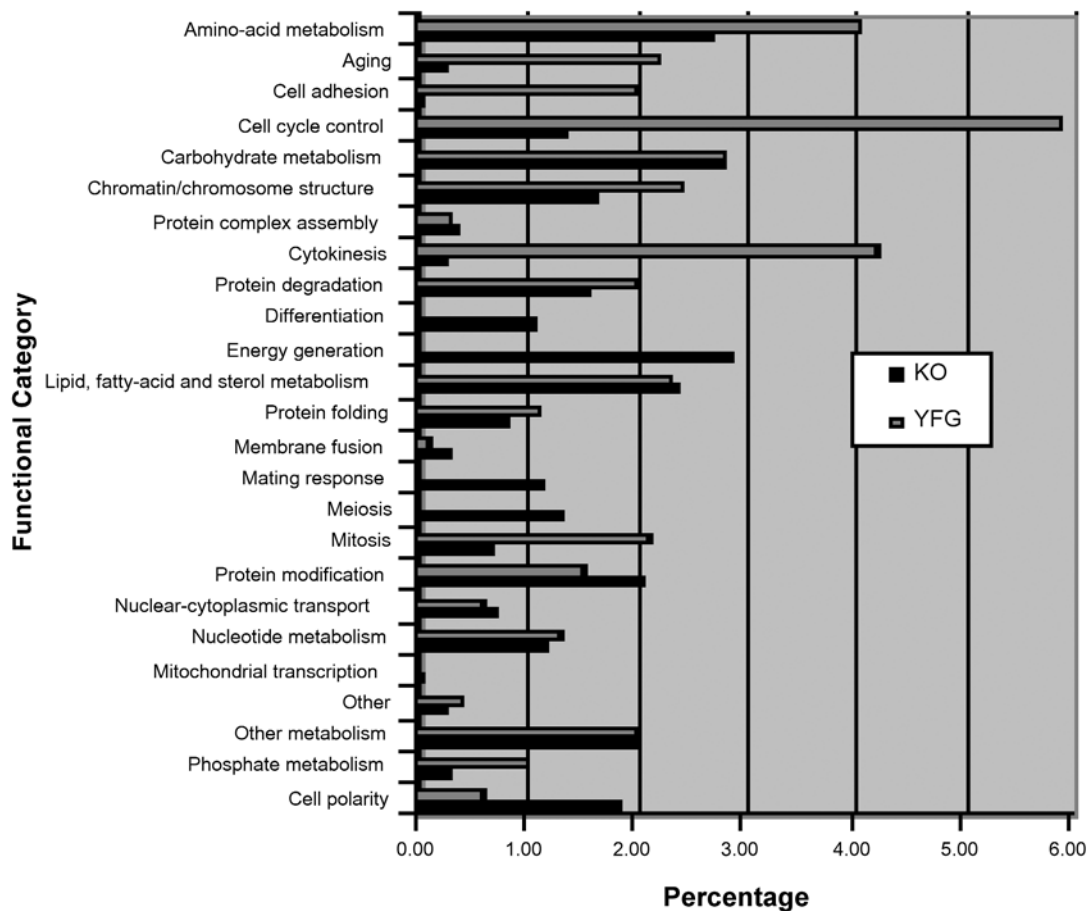


Fig. 3. Enrichment of genes in specific functional categories in a synthetic lethal screen using the YGDS (hypothetical example). After completing a synthetic lethal screen with the YGDS (labeled YFG), identified yeast genes are grouped in functional categories based on known or predicted functions. The percentage of genes in each functional category from the synthetic lethal screen (YFG) is then compared directly with the percentage of genes in each functional category across the entire knockout (KO) collection, indicating selective enrichment in specific functional categories.

genome (such as those that encode caspases involved in apoptosis or growth factors). Despite this caveat, our finding that yeast genes with human orthologs were enriched in our screens to levels significantly higher than would have been predicted by chance suggests the possibility that with yeast we might have discovered cell autonomous biological response pathways that are relevant to neurodegeneration. Ultimately, the validity of using yeast as a model organism for studying pathogenic mechanisms of neurodegeneration will only be established by performing complementary approaches in more physiologically relevant models of neurodegeneration.

Yeast as a Tool for Drug Screening

We have recently initiated large-scale drug-screening efforts in our yeast models of amyloid for-

mation to identify therapeutic compounds for the treatment of neurodegenerative diseases. In comparison to mammalian cells, yeast has numerous advantages at the early stages of the drug development process. The low cost of growing yeast cells, a short doubling time, the ease of genetic manipulation, and high resistance to solvents collectively make *S. cerevisiae* an attractive organism for performing cell-based high-throughput screens. One potential limiting factor for the use of yeast as an assay system is the presence of the cell wall, which might be impermeable to some organic molecules. Nevertheless, this undesirable attribute can be eliminated by chemical or genetic means, by increasing the permeability of yeast cells or by reducing the capability of the yeast cells to export drugs. It is important to stress that drug-screening assays in yeast are considered only as primary screening assays that will be comple-

mented by follow-up assays in more physiologically relevant models to allow the successful identification and confirmation of novel lead compounds.

The use of the simple model organism yeast has an illustrious history of leading to significant advances in our knowledge of human diseases, even in cases in which it was initially difficult to conceive of the utility of this organism (e.g., cancer). The pathogenic mechanisms that underlie neurodegeneration are not well understood, and novel approaches, such as those being performed in yeast, are desperately needed. Even more importantly, genetic approaches in yeast might identify novel genes not known previously to play any role in neurodegeneration, as well as genes whose products might prove to be attractive therapeutic targets for the treatment of neurodegenerative diseases.

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References

- Abeliovich A., Schmitz Y., Farinas I., Choi-Lundberg D., Ho W. H., Castillo P. E., et al. (2000) Mice lacking alpha-synuclein display functional deficits in the nigrostriatal dopamine system. *Neuron* **25**, 239–252.
- Ahn B. H., Rhim H., Kim S. Y., Sung Y. M., Lee M. Y., Choi J. Y., et al. (2002) Alpha-Synuclein interacts with phospholipase D isozymes and inhibits pervanadate-induced phospholipase D activation in human embryonic kidney-293 cells. *J. Biol. Chem.* **277**, 12334–12342.
- Babcock M., de Silva D., Oaks R., Davis-Kaplan S., Jiralerspong S., Montermini L., et al. (1997) Regulation of mitochondrial iron accumulation by Yfh1p, a putative homolog of frataxin. *Science* **276**, 1709–1712.
- Bence N. F., Sampat R. M., and Kopito R. R. (2001) Impairment of the ubiquitin-proteasome system by protein aggregation. *Science* **292**, 1552–1555.
- Birrell G. W., Giaever G., Chu A. M., Davis R. W., and Brown J. M. (2001) A genome-wide screen in *Saccharomyces cerevisiae* for genes affecting UV radiation sensitivity. *Proc. Natl. Acad. Sci. USA* **98**, 12608–12613.
- Bruijn L. I., Houseweart M. K., Kato S., Anderson K. L., Anderson S. D., Ohama E., et al. (1998) Aggregation and motor neuron toxicity of an ALS-linked SOD1 mutant independent from wild-type SOD1. *Science* **281**, 1851–1854.
- Campuzano V., Montermini L., Molto M. D., Pianese L., Cossee M., Cavalcanti F., et al. (1996) Friedreich's ataxia: autosomal recessive disease caused by an intronic GAA triplet repeat expansion. *Science* **271**, 1423–1427.
- Cavadini P., Gellera C., Patel P. I., and Isaya G. (2000) Human frataxin maintains mitochondrial iron homeostasis in *Saccharomyces cerevisiae*. *Hum. Mol. Genet.* **9**, 2523–2530.
- Chan T. F., Carvalho J., Riles L., and Zheng X. F. (2000) A chemical genomics approach toward understanding the global functions of the target of rapamycin protein (TOR). *Proc. Natl. Acad. Sci. USA* **97**, 13227–13232.
- Clayton D. F. and George J. M. (1999) Synucleins in synaptic plasticity and neurodegenerative disorders. *J. Neurosci. Res.* **58**, 120–129.
- Cleveland D. W. (1999) From Charcot to SOD1: mechanisms of selective motor neuron death in ALS. *Neuron* **24**, 515–520.
- Cleveland D. W. and Rothstein J. D. (2001). From Charcot to Lou Gehrig: deciphering selective motor neuron death in ALS. *Nat. Rev. Neurosci.* **2**, 806–819.
- Conway K. A., Harper J. D., and Lansbury P. T., Jr. (2000a) Fibrils formed in vitro from alpha-synuclein and two mutant forms linked to Parkinson's disease are typical amyloid. *Biochemistry* **39**, 2552–2563.
- Conway K. A., Lee S. J., Rochet J. C., Ding T. T., Williamson R. E., and Lansbury P. T., Jr. (2000b) Acceleration of oligomerization, not fibrillization, is a shared property of both alpha-synuclein mutations linked to early-onset Parkinson's disease: implications for pathogenesis and therapy. *Proc. Natl. Acad. Sci. USA* **97**, 571–576.
- Corson L. B., Folmer J., Strain J. J., Culotta V. C., and Cleveland D. W. (1999) Oxidative stress and iron are implicated in fragmenting vacuoles of *Saccharomyces cerevisiae* lacking Cu,Zn-superoxide dismutase. *J. Biol. Chem.* **274**, 27590–27596.
- Corson L. B., Strain J. J., Culotta V. C., and Cleveland D. W. (1998) Chaperone-facilitated copper binding is a property common to several classes of familial amyotrophic lateral sclerosis-linked superoxide dismutase mutants. *Proc. Natl. Acad. Sci. USA* **95**, 6361–6366.
- Davidson W. S., Jonas A., Clayton D. F., and George J. M. (1998) Stabilization of alpha-synuclein secondary structure upon binding to synthetic membranes. *J. Biol. Chem.* **273**, 9443–9449.
- Engelender S., Kaminsky Z., Guo X., Sharp A. H., Amaravi R. K., Kleiderlein J. J., et al. (1999) Synphilin-1 associates with alpha-synuclein and promotes the formation of cytosolic inclusions. *Nat. Genet.* **22**, 110–114.
- Feany M. B. and Bender W. W. (2000) A *Drosophila* model of Parkinson's disease. *Nature* **404**, 394–398.
- Fernandez-Funez P., Nino-Rosales M. L., de Gouyon B., She W. C., Luchak J. M., Martinez P., et al. (2000). Identification of genes that modify ataxin-1-induced neurodegeneration. *Nature* **408**, 101–106.
- Foury F. and Cazzalini O. (1997) Deletion of the yeast homologue of the human gene associated with Friedreich's ataxia elicits iron accumulation in mitochondria. *FEBS Lett.* **411**, 373–377.

- Fridovich I. (1995) Superoxide radical and superoxide dismutases. *Annu. Rev. Biochem.* **64**, 97–112.
- George J. M., Jin H., Woods W. S., and Clayton D. F. (1995) Characterization of a novel protein regulated during the critical period for song learning in the zebra finch. *Neuron* **15**, 361–372.
- Giaever G., Chu A. M., Ni L., Connelly C., Riles L., Veronneau S., et al. (2002). Functional profiling of the *Saccharomyces cerevisiae* genome. *Nature* **418**, 387–391.
- Giasson B. I., Duda J. E., Quinn S. M., Zhang B., Trojanowski J. Q., and Lee V. M. (2002) Neuronal alpha-synucleinopathy with severe movement disorder in mice expressing A53T human alpha-synuclein. *Neuron* **34**, 521–533.
- Goedert M. (2001) Alpha-synuclein and neurodegenerative diseases. *Nat. Rev. Neurosci.* **2**, 492–501.
- Goffeau A., Barrell B. G., Bussey H., Davis R. W., Dujon B., Feldmann H., et al. (1996) Life with 6000 genes. *Science* **274**, 546, 563–547.
- Greenfield J. P., Xu H., Greengard P., Gandy S., and Seeger M. (1999) Generation of the amyloid-beta peptide N terminus in *Saccharomyces cerevisiae* expressing human Alzheimer's amyloid-beta precursor protein. *J. Biol. Chem.* **274**, 33843–33846.
- Hardy J. and Selkoe D. J. (2002) The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* **297**, 353–356.
- The Huntington's Disease Collaborative Research Group (1993) A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell* **72**, 971–983.
- Jenco J. M., Rawlingson A., Daniels B., and Morris A. J. (1998) Regulation of phospholipase D2: selective inhibition of mammalian phospholipase D isoenzymes by alpha- and beta-synucleins. *Biochemistry* **37**, 4901–4909.
- Jensen P. H., Nielsen M. S., Jakes R., Dotti C. G., and Goedert M. (1998) Binding of alpha-synuclein to brain vesicles is abolished by familial Parkinson's disease mutation. *J. Biol. Chem.* **273**, 26292–26294.
- Johnston J. A., Ward C. L., and Kopito R. R. (1998) Aggresomes: a cellular response to misfolded proteins. *J. Cell Biol.* **143**, 1883–1898.
- Kahle P. J., Neumann M., Ozmen L., Muller V., Jacobsen H., Schindzielorz A., et al. (2000) Subcellular localization of wild-type and Parkinson's disease-associated mutant alpha-synuclein in human and transgenic mouse brain. *J. Neurosci.* **20**, 6365–6373.
- Kazemi-Esfarjani P. and Benzer S. (2000) Genetic suppression of polyglutamine toxicity in *Drosophila*. *Science* **287**, 1837–1840.
- Knight S. A., Kim R., Pain D., and Dancis A. (1999) The yeast connection to Friedreich ataxia. *Am. J. Hum. Genet.* **64**, 365–371.
- Kopito R. R. (2000) Aggresomes, inclusion bodies and protein aggregation. *Trends Cell Biol.* **10**, 524–530.
- Koutnikova H., Campuzano V., Foury F., Dolle P., Cazzalini O., and Koenig M. (1997) Studies of human, mouse and yeast homologues indicate a mitochondrial function for frataxin. *Nat. Genet.* **16**, 345–351.
- Krobitsch S. and Lindquist S. (2000) Aggregation of huntingtin in yeast varies with the length of the polyglutamine expansion and the expression of chaperone proteins. *Proc. Natl. Acad. Sci. USA* **97**, 1589–1594.
- Lindquist S., Krobitsch S., Li L., and Sondheimer N. (2001) Investigating protein conformation-based inheritance and disease in yeast. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **356**, 169–176.
- Lotharius J. and Brundin P. (2002) Pathogenesis of Parkinson's disease: dopamine, vesicles and alpha-synuclein. *Nat. Rev. Neurosci.* **3**, 932–942.
- Martzen M. R., McCraith S. M., Spinelli S. L., Torres F. M., Fields S., Grayhack E. J., and Phizicky E. M. (1999) A biochemical genomics approach for identifying genes by the activity of their products. *Science* **286**, 1153–1155.
- Maslah E., Rockenstein E., Veinbergs I., Mallory M., Hashimoto M., Takeda A., et al. (2000) Dopaminergic loss and inclusion body formation in alpha-synuclein mice: implications for neurodegenerative disorders. *Science* **287**, 1265–1269.
- McLean P. J., Kawamata H., and Hyman B. T. (2001) Alpha-Synuclein-enhanced green fluorescent protein fusion proteins form proteasome sensitive inclusions in primary neurons. *Neuroscience* **104**, 901–912.
- Meriin A. B., Zhang X., He X., Newnam G. P., Chernoff Y. O., and Sherman M. Y. (2002) Huntington toxicity in yeast model depends on polyglutamine aggregation mediated by a prion-like protein Rnq1. *J. Cell Biol.* **157**, 997–1004.
- Muchowski P. J. (2002) Protein misfolding, amyloid formation, and neurodegeneration: a critical role for molecular chaperones? *Neuron* **35**, 9–12.
- Muchowski P. J., Ning K., D'Souza-Schorey C., and Fields S. (2002) Requirement of an intact microtubule cytoskeleton for aggregation and inclusion body formation by a mutant huntingtin fragment. *Proc. Natl. Acad. Sci. USA* **99**, 727–732.
- Muchowski P. J., Schaffar G., Sittler A., Wanker E. E., Hayer-Hartl M. K., and Hartl F. U. (2000) Hsp70 and hsp40 chaperones can inhibit self-assembly of polyglutamine proteins into amyloid-like fibrils. *Proc. Natl. Acad. Sci. USA* **97**, 7841–7846.
- Nishida C. R., Gralla E. B., and Valentine J. S. (1994) Characterization of three yeast copper-zinc superoxide dismutase mutants analogous to those coded for in familial amyotrophic lateral sclerosis. *Proc. Natl. Acad. Sci. USA* **91**, 9906–9910.
- Outeiro T. F. and Lindquist S. (2003) Yeast cells provide insight into alpha-synuclein biology and pathobiology. *Science* **302**, 1772–1775.
- Perrin R. J., Woods W. S., Clayton D. F., and George J. M. (2000) Interaction of human alpha-synuclein and Parkinson's disease variants with phospholipids. Structural analysis using site-directed mutagenesis. *J. Biol. Chem.* **275**, 34393–34398.
- Puccio H. and Koenig M. (2000) Recent advances in the molecular pathogenesis of Friedreich ataxia. *Hum. Mol. Genet.* **9**, 887–892.

- Rabizadeh S., Gralla E. B., Borchelt D. R., Gwinn R., Valentine J. S., Sisodia S., et al. (1995) Mutations associated with amyotrophic lateral sclerosis convert superoxide dismutase from an antiapoptotic gene to a proapoptotic gene: studies in yeast and neural cells. *Proc. Natl. Acad. Sci. USA* **92**, 3024–3028.
- Rochet J. C. and Lansbury P. T., Jr. (2000) Amyloid fibrillogenesis: themes and variations. *Curr. Opin. Struct. Biol.* **10**, 60–68.
- Ross C. A. (2002) Polyglutamine pathogenesis: emergence of unifying mechanisms for Huntington's disease and related disorders. *Neuron* **35**, 819–822.
- Ross-Macdonald P., Coelho P. S., Roemer T., Agarwal S., Kumar A., Jansen R., et al. (1999) Large-scale analysis of the yeast genome by transposon tagging and gene disruption. *Nature* **402**, 413–418.
- Schwikowski B., Uetz P., and Fields S. (2000) A network of protein-protein interactions in yeast. *Nat. Biotechnol.* **18**, 1257–1261.
- Sharon R., Goldberg M. S., Bar-Josef I., Betensky R. A., Shen J., and Selkoe D. J. (2001) Alpha-Synuclein occurs in lipid-rich high molecular weight complexes, binds fatty acids, and shows homology to the fatty acid-binding proteins. *Proc. Natl. Acad. Sci. USA* **98**, 9110–9115.
- Sherman F. (1991) Getting started with yeast. *Methods Enzymol.* **194**, 3–21.
- Sherman M. Y. and Goldberg A. L. (2001) Cellular defenses against unfolded proteins: a cell biologist thinks about neurodegenerative diseases. *Neuron* **29**, 15–32.
- Shimohata T., Sato A., Burke J. R., Strittmatter W. J., Tsuji S., and Onodera O. (2002) Expanded polyglutamine stretches form an 'aggresome'. *Neurosci. Lett.* **323**, 215–218.
- Singleton A. B., Farrer M., Johnson J., Singleton A., Hague S., Kachergus J., et al. (2003) Alpha-synuclein locus triplication causes Parkinson's disease. *Science* **302**, 841.
- Steinmetz L. M., Scharfe C., Deutschbauer A. M., Mokranjac D., Herman Z. S., Jones T., et al. (2002) Systematic screen for human disease genes in yeast. *Nat. Genet.* **31**, 400–404.
- Tobin A. J. and Signer E. R. (2000) Huntington's disease: the challenge for cell biologists. *Trends Cell Biol.* **10**, 531–536.
- Uetz P., Giot L., Cagney G., Mansfield T. A., Judson R. S., Knight J. R., et al. (2000) A comprehensive analysis of protein-protein interactions in *Saccharomyces cerevisiae*. *Nature* **403**, 623–627.
- Uptain S. M. and Lindquist S. (2002) Prions as protein-based genetic elements. *Annu. Rev. Microbiol.* **56**, 703–741.
- van der Putten H., Wiederhold K. H., Probst A., Barbieri S., Mistl C., Danner S., et al. (2000) Neuropathology in mice expressing human alpha-synuclein. *J. Neurosci.* **20**, 6021–6029.
- Volles M. J. and Lansbury P. T., Jr. (2002) Vesicle permeabilization by protofibrillar alpha-synuclein is sensitive to Parkinson's disease-linked mutations and occurs by a pore-like mechanism. *Biochemistry* **41**, 4595–4602.
- Volles M. J., Lee S. J., Rochet J. C., Shtilerman M. D., Ding T. T., Kessler J. C., and Lansbury P. T., Jr. (2001) Vesicle permeabilization by protofibrillar alpha-synuclein: implications for the pathogenesis and treatment of Parkinson's disease. *Biochemistry* **40**, 7812–7819.
- Waelter S., Boeddrich A., Lurz R., Scherzinger E., Lueder G., Lehrach H., and Wanker E. E. (2001) Accumulation of mutant huntingtin fragments in aggresome-like inclusion bodies as a result of insufficient protein degradation. *Mol. Biol. Cell* **12**, 1393–1407.
- Willingham S., Outeiro T. F., DeVit M. J., Lindquist S. L., and Muchowski P. J. (2003) Yeast genes that enhance the toxicity of a mutant huntingtin fragment or alpha-synuclein. *Science* **302**, 1769–1772.
- Wilson R. B. and Roof D. M. (1997) Respiratory deficiency due to loss of mitochondrial DNA in yeast lacking the frataxin homologue. *Nat. Genet.* **16**, 352–357.
- Winzeler E. A., Shoemaker D. D., Astromoff A., Liang H., Anderson K., Andre B., et al. (1999) Functional characterization of the *S. cerevisiae* genome by gene deletion and parallel analysis. *Science* **285**, 901–906.
- Zhang W., Espinoza D., Hines V., Innis M., Mehta P., and Miller D. L. (1997) Characterization of beta-amyloid peptide precursor processing by the yeast Yap3 and Mkc7 proteases. *Biochim. Biophys. Acta* **1359**, 110–122.
- Zoghbi H. Y. and Orr H. T. (2000) Glutamine repeats and neurodegeneration. *Annu. Rev. Neurosci.* **23**, 217–247.

