Modelling Neurodegeneration in Saccharomyces Cerevisiae: Why Cook with Baker's Yeast?

Citation: Khurana, Vikram, and Susan Lindquist. "Modelling neurodegeneration in Saccharomyces cerevisiae: why cook with baker's yeast?" Nature Reviews Neuroscience 11.6 (2010): 436-449.

As Published: http://dx.doi.org/10.1038/nrn2809

Publisher: Nature Publishing Group

Persistent URL: http://hdl.handle.net/1721.1/69914

Version: Author's final manuscript: final author's manuscript post peer review, without publisher's formatting or copy editing

Perspective Opinion Article ***Nature Reviews Neuroscience***

Modelling neurodegeneration in *S. cerevisiae*: Why cook with Baker's yeast?

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Abstract

In aging populations like our own, neurodegenerative diseases continue to increase in prevalence, exacting an enormous toll on individuals and their communities. Multiple complementary experimental approaches are needed to elucidate the mechanisms underlying these complex diseases and develop novel therapeutics. Here we describe why the budding yeast, *Saccharomyces cerevisiae*, plays a unique role in the neurodegeneration armamentarium. As the best-understood and most readily analyzed eukaryotic organism, *S. cerevisiae* is delivering mechanistic insights into cell-autonomous mechanisms of neurodegeneration at an interactome-wide scale.

Introduction

Neurodegenerative diseases are among the most pressing public health challenges facing the aging populations of developed nations. For more than a century, the study of

neurodegeneration was confined to relating the devastating clinical phenotype of these diseases to their post-mortem neuropathology. While the neuropathologic observations have been instrumental in identifying pathologic proteinacious aggregates and patterns of differential neuronal vulnerability, they cannot distinguish causal from epiphenomenenal factors in disease pathogenesis. In the last 15 years, we have gained tremendous insight into disease aetiology with the identification of numerous disease-causing mutations. Neuropathologic observations and molecular genetics have been mutually beneficial. For example, the identification of mutations in genes that encode proteins that aggregate in neurodegenerative diseases has causally tied these proteins to the disease process. The neuropathologic observation that the same proteins aggregate in the familial and sporadic forms of certain neurodegenerative diseases has strongly suggested that studying the consequences of rare gene mutations has relevance for the more common sporadic disease.

Perhaps most importantly, the identification of disease-causing mutations and misfolded proteins has enabled the creation of cellular and animal models of neurodegenerative diseases. The remarkable homology between organisms separated in evolution by hundreds of millions of years has provided good reasons to believe that insights gained by study of these model systems will be relevant to the study and treatment of human disease. For example, identification and characterization of an upstream causative role for beta-amyloid production in Alzheimer's disease (AD) has been a concerted effort in which neuropathology, biochemistry, human genetics and work within model systems including fruit fly, worm and mouse have each played key roles. It is becoming clear that multiple complementary approaches, each with advantages and disadvantages, will be needed to make significant strides toward developing therapies.

In a research community becoming accustomed to a plethora of different model systems, it nevertheless remains surprising to many that complex disease processes such as those contributing to neurodegenerative diseases can be modelled productively in the budding yeast *Saccharomyces cerevisiae*, best known in the general community for its role in the leavening of dough and brewing of beer. In this article we aim to show that yeast has a unique role in the understanding of neurodegenerative disease. Yeast disease models capture key aspects of cellular pathology and, through the use of high-throughput screens, are providing novel gene-environment connections and therapeutic targets at an unprecedented scale.

The yeast model system

Two general characteristics confer suitability upon any model system for the study of human disease: it must be relevant and it must be readily amenable to analysis. *S. cerevisiae* rises to both challenges. The relevance to human disease is well established by its conserved genome and cellular biology. Amenability to analysis relates to its genetic tractability, scalability and short generation time. These features have led directly to the development of an ever-increasing number of high-throughput tools[1]. While we focus exclusively on *S. cerevisiae*, another yeast species *Schizosaccharomyces pombe* is fast becoming a powerful model in its own right [2, 3].

Relevance to neurodegeneration. In 1996, *S. cerevisiae* became the first eukaryote to have its 1.3×10^7 base pairs-long genome sequenced. By comparison, the human genome has 3.08×10^9 base pairs but only 3 to 5 times as many genes. To date, approximately 6600 open

reading frames (ORFs; protein-encoding genomic sequences) have been annotated, with more than 80% functionally characterized[4]. At least 60% of yeast genes have statistically robust human homologs or at least one conserved domain with human genes[1, 5]. In addition, more than 25% of positionally cloned human disease genes have a close yeast homolog[6, 7]. Genomic homology explains the conservation of fundamental cell biologic processes between yeast and mammalian cells. Yeast cells, like mammalian ones, are eukaryotic, distinguished from prokaryotes such as bacteria by the presence of membrane-bound organelles, including a nucleus. Yeast cells recapitulate fundamental aspects of eukaryotic biology including a distinctive process of cell division and genetic transmission, transcriptional regulation, biogenesis and function of cellular organelles, protein targeting and secretion, cytoskeletal dynamics and regulation, and cellular metabolism.

The conservation of homologous genes fulfilling similar functions has been a recurrent theme in eukaryotic cell biology, often with interchangeability of yeast and mammalian homologs (genetic complementation). Homology to a yeast gene has often provided the first clue to the function of many higher eukaryotic genes.

A few conserved aspects of cellular biology, which have benefited from rigorous molecular dissection in yeast, warrant particular mention in the context of neurodegenerative disease (Fig. 1). The most common neurodegenerative diseases, including AD and Parkinson's disease (PD), are associated with intracellular proteinacious aggregates. Multiple lines of evidence intimately associate protein misfolding, oligomerization and aggregation with neurodegeneration. These processes are readily studied in yeast because there is high conservation of the cellular protein quality system. The archetypal protein-folding diseases are caused by prions, infectious protein particles that misfold and aggregate within neurons

and lead to neurodegeneration. Yeast prions were identified as non-Mendelian elements of inheritance, which, like mammalian prions, altered cellular phenotype via a protein-only mode of transmission[8]. They have different functions, different cellular locations and no sequence similarity to their mammalian counterparts. However, the rigorous genetic and biochemical analysis in yeast was seminal in establishing the protein-only hypothesis of prion infectivity and the different conformational states of prion proteins, one of which is infectious and templates its conformation to non-prion conformers. Yeast amyloid exhibits similar biochemical properties to amyloid in neurodegenerative diseases, including recognition by Congo Red and Thioflavin T, beta strands running perpendicular to the fiber axis, and the formation of molten pre-amyloid oligomeric species that react with the same conformation-specific antibody. Yeast has shed light on other processes including the molecular mechanisms by which prions form different strains, multiple infectious conformations that encode unique biological phenotypes, and the mechanism through which prion species barriers are established and overcome[9].

Mitochondrial dysfunction and oxidative stress are heavily implicated in neurodegeneration. In yeast, as in mammalian cells, the central organelle for the production of reactive oxygen species (ROS) is the mitochondrion. The ability of yeast to grow in fermentative states allows for the analysis of mitochondrial defects that would be lethal in mammalian cells. Studies in yeast have yielded fundamental insights into mammalian mitochondrial biology including the discovery of genes that regulate fission and fusion of mitochondria. Genetic defects in this machinery have now been causally linked to neurodegenerative disease[10].

The secretory pathway, through which proteins are translocated from endoplasmic

reticulum (ER) to Golgi and then trafficked in vesicles to the plasma membrane, is of particular importance in neurons that need to transport proteins over long distances to nerve terminals and that release neurotransmitters via vesicular fusion. Our understanding of the molecular mechanisms of this pathway owe a great deal to yeast, in which screening for mutants that cause accumulation of secretory proteins resulted in the discovery of 23 *SEC* genes, encoding the central components of the secretory and vesicular trafficking machinery[11]. Yeast has homologs of synaptobrevin, syntaxin and SNAP-25 among other critical mammalian components of this pathway[12]. Importantly, ER stress caused by accumulation of misfolded proteins in vesicular trafficking has been heavily implicated in neurodegeneration and cerebral white matter disease[13-15]. In Box 2 we present data that identify this pathway as a critical mediator of toxicity in synucleinopathies, neurodegenerative diseases associated with neuronal and/or glial accumulation of aggregated α-synuclein (α-syn).

As a final example, we note that yeast have conserved mechanisms of cell death and survival that are likely to be relevant to neuronal loss. Both apoptotic and non-apoptotic cell death mechanisms have been implicated in neurodegeneration. In addition, aberrant re-activation of highly conserved cell-cycle mechanisms within postmitotic neurons may lead to apoptosis in some contexts[16]. As in mammalian cells, an apoptosis-like process has been described in yeast that involves chromatin condensation, altered mitochondrial membrane potential, release of cytochrome *c*, exposure of phophatidylserine at the plasma membrane and labelling by TUNEL (Terminal deoxynucleotidyl transferase dUTP nick end labeling) staining[17]. There is accumulating evidence that fundamental molecular mechanisms of programmed cell death (PCD) may be conserved in yeast. Although the existence of a

programmed cell death pathway in a unicellular organism may seem surprising, there are benefits in a clonal population for those cells that are accumulating oxidative damage to undergo cell death rather than to deprive genetically identical neighbouring cells of nutrients[18]. Experiments on yeast "apoptosis" illustrates how molecular analysis of a process in yeast can be productive even when yeast homologs for particular genes do not exist. Despite the presence of apoptotic mediators in yeast cells, they lack the mammalian Bcl/Bax family of apoptotic regulators. Nevertheless, heterologous expression of mammalian Bcl/Bax family members in yeast reveals conserved modulation of cell survival. When Bax protein is expressed in yeast, for example, it localizes to the yeast mitochondria and promotes cell death. Indeed, genetic and biophysical analysis in yeast has yielded important insights into mammalian Bax function[19]. The conserved function exists presumably because yeast possess "Bcl-2-like" proteins, with divergent sequences from their mammalian counterparts, that are functional homologs, such as the yeast metacaspase YCA1, or that share a common function despite different evolutionary origin.

Enormous attention has been directed recently to the potential role of autophagy in neuronal survival, putatively by degradation of misfolded proteins and elimination of damaged organelles. Genetic analysis in yeast played a pivotal role in identifying the effector machinery of autophagy, comprising the so-called ATG proteins downstream of the target of rapamycin (TOR) kinase, now known to be highly conserved in mammalian cells[20]. Deletion of ATG proteins in postmitotic mammalian neurons results in the accumulation of misfolded proteins, and genetic and pharmacologic inhibition of TOR is neuroprotective in a number of neurodegeneration models[21].

Amenability to analysis. As a model system, yeast offers the advantage of a short generation time (1.5-3 hours), and grows in a highly reproducible and genetically stable way. As yeast is unicellular, it is also a scalable system and thus suited for high-throughput genetic and small molecule screens. Most important is its genetic tractability, which relies on the ease of DNA transformation and the efficiency with which homologous recombination occurs[22, 23]. This facilitates both the replacement of any gene with a mutant allele and the recovery of any mutation created *in vivo*. Reduced genetic redundancy means the effect of gene knockout or replacement genetic analysis is less likely to be masked by closely related products taking over the target's role, a serious drawback of equivalent experiments in more complex systems. Although it has a diploid life cycle, early genetic manipulations allowed stable vigorous propagation in the haploid state and strains with distinct phenotypes could be mated. The ease of meiotic segregation analysis enables the recovery and phenotypic characterization of recessive mutants, tests of allelic complementation and recombination, immediate means to distinguish between simple and complex traits, and the determination of epistatic genetic relationships.

The yeast toolbox. Genetic tractability, combined with a highly collaborative community of yeast researchers, has led to a vast array of analytic tools. A phenotype resulting from a genetic, environmental or chemical perturbation can now be analyzed by genome-wide genetic interaction screens, transcriptional profiling, proteomic analysis, lipidomic analysis, small molecule and chemical-genetic analysis, and phenotype microarrays (Table 1). *S. cerevesiae* is indeed establishing an integrated "-omic"s picture (the "interactome") of the genes, transcripts, proteins, and metabolites to describe normal and perturbed cellular

functioning. Genetic tractability combined with genomic stability and offer significant advantages over transformed mammalian cell culture lines. Genomic instability and non-physiologic perturbation of cell survival pathways may be undesirable features of a model system for neurodegenerative diseases in particular.

Phenotypes can now be screened against genome-wide deletion, reduced expression or over-expression libraries. The latter include libraries of plasmids for manipulating the expression of every open reading frame of the yeast genome under the expression of different promoters (Table 1). Automated screening of a phenotype against the entire over-expression library can be accomplished in a matter of weeks. Hundreds of genome-wide screens for synthetic lethal genetic interactions (combinations of two mutations which themselves have little or no phenotype, but which together are severely toxic or inviable) have been used extensively to identify genes whose products buffer one another's functions or impinge on the same pathway. To date ~37,000 genetic interactions have been mapped, although it is estimated that a global network will eventually contain ~200,000 synthetic lethal interactions[35]. In addition, several large-scale proteomics projects, by both two-hybrid analysis and by high-throughput mass spectroscopy, have yielded ~45,000 protein-protein interactions[37, 38] and thousands of expression profiles have been generated with diverse genetic, chemical, and environmental perturbations. The wealth of interaction data have been compiled online in publicly available databases (Table 2).

Limitations. As a unicellular organism with a cell wall, the most obvious and important limitation of yeast as a model system is for studying aspects of neurodegenerative disease that rely on multicellularity and the interactions between cells. These important aspects of

disease biology include immune and inflammatory responses, synaptic transmission and glia-neuronal interactions to list just three.

Mammalian cells have diversified to include cellular specializations without homology in yeast. Yeast mitochondria, for example, lack a typical complex I. Yeast cells carry out the functions of complex I (such as oxidation of NADH and reduction of quinone) with a structurally much simpler complex called NDH-2 that is not sensitive to mammalian complex I inhibitors. Although the basic elements of the unfolded protein response to ER stress are conserved in yeast, it is far more complex in mammalian cells[24]. Many neuronal specializations that are likely to be of great importance to neurodegeneration - axonal transport, neurotransmitter release, myelination among others – cannot be recapitulated in yeast. Nevertheless, fundamental aspects of these biologic functions may be conserved in yeast. For example, although yeast cells do not release neurotransmitters, they do traffic proteins in vesicles and have conserved endo- and exocytic mechanisms; and, although yeast cells do not produce myelin, they have substantially conserved lipid biosynthesis pathways. As mentioned above, heterologous expression of a protein that is absent in yeast can still be highly informative as the protein may have many conserved protein interactions. Still, the important biological differences between yeast and complex diseases require that insights gained from the yeast system are validated in neuronal model systems.

Creating yeast models of a neurodegenerative disease

Modelling human disease in yeast follows one of two general approaches, depending on whether or not a yeast homolog exists (Fig. 2). When a human disease-related gene has a yeast homolog, the gene can be disrupted or over-expressed to determine the loss- or gain-of-

function phenotypes, respectively[8]. Clear yeast homologs exist for many genes associated with neurodegeneration (a non-exhaustive list is provided in Table 3). This modelling approach has already been very productive. For example, Friedreich's ataxia is an autosomal recessive neurodegenerative ataxia caused by a substantial reduction in levels of frataxin, encoded by the gene *FRDA*. Studies of the yeast *FRDA* homolog *YFH1* have been instrumental in determining the function of frataxin. Yeast with YFH1 deletions are unable to grow on non-fermentable carbon sources, indicating mitochondrial dysfunction and defective oxidative phosphorylation, accompanied by mitochondrial iron overload, increased ROS, hypersensitivity to oxidative stress, and defective synthesis of iron-sulfur cluster enzymes including aconitase. Importantly, human *FRDA* rescues these phenotypes. These findings in yeast motivated assays for similar phenotypes in mouse models and in fibroblasts from patients, which have been confirmatory. Whereas the exact role of each of these cellular defects in causing pathology is debated, the anti-oxidant idebenone has shown promise for both neurologic and cardiac manifestations in early clinical trials[25, 26].

For human disease-related genes that do not have a yeast homolog and for which the disease process is clearly a toxic gain of RNA or protein function, the human gene is expressed in yeast and screens are designed against any relevant phenotypes that result from this expression. Typically, neurodegenerative diseases in this category are autosomal dominant and involve aggregation of the protein encoded by the mutated gene, strongly implicating protein misfolding and the formation of a toxic protein species (whether large aggregates or oligomers) in disease pathogenesis. Diseases modelled in this way include Huntington's and other polyglutamine diseases, synucleinopathies including Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis, prion diseases and tauopathies

(Table 4). Creating a cellular or animal model by ectopic expression of the implicated proteins is justifiable if over-expression recapitulates key features of the disease, if knocking out or reducing gene dosage does not recapitulate these features and, perhaps most convincingly, if the human disease can be caused by inheriting extra copies of the encoding gene. In the following sections and Boxes 1 and 2 we describe two yeast models that recapitulate key cellular pathologies of the human disease that have led to important mechanistic insights validated in neuronal model systems.

Yeast model of polyglutamine (polyQ) expansion disorders **(Box 1).** Polyglutamine expansion disorders are autosomal dominant disorders caused by a CAG repeat expansion within the open reading frame, encoding a protein with an expanded polyQ tract. A polyQ tract must exceed a critical length, usually ~35, to initiate disease, with longer tracts generally causing earlier disease onset. This disease category includes spinobulbar muscular atrophy, dentatorubral-pallidoluysian atrophy and a number of spinocerebellar ataxias, as well as the prototypic disease in this group, Huntington's disease (HD)[26]. HD is characterized by hyperkinetic movements, psychosis and cognitive dysfunction and is caused by polyQ expansion within the N-terminal end of the Huntingtin (Htt) protein, leading to loss of striatal medium spiny neurons and cortical neurons. Htt exon 1 (Htt-ex1) is found in the neuronal aggregates of HD and its over-expression is sufficient to produce neurodegeneration in mouse models[27]. The yeast model was made by over-expressing fragments of Htt exon I, (Htt-ex1) followed by polyQ tracts of different lengths. Phenotypes include polyQ length-dependent Htt-ex1 aggregation and the formation of higher order complexes[28-31], impaired growth accompanied by mitochondrial dysfunction and oxidative stress, apoptosis-like DNA

fragmentation[32, 33], transcriptional dysregulation[28], and a dramatic deficit of endoplasmic reticulum-associated degradation (ERAD) associated with an unfolded protein response[34].

Yeast model of synucleinopathy **(Box 2).** Aggregated α-Synuclein (α-Syn), a small 14-kDa protein principally associated with phospholipids in membranes and presynaptic vesicles in neurons, is observed in PD, dementia with Lewy bodies, multiple system atrophy and neurodegeneration with brain iron accumulation type1. PD is characterized clinically by parkinsonism (bradykinesia, rigidity and resting tremor) and responsiveness to L-DOPA therapy, and pathologically by neuronal α-Syn inclusions known as Lewy bodies (LBs) and prominent loss of midbrain dopamine (DA) and other neuronal populations. ~5 per cent of DOPA-responsive parkinsonism is associated with mutations at various *PARK* loci causing parkinsonism with Mendelian inheritance. Mutations at the *SNCA* (*PARK1*) locus that encodes α-Syn include point mutations and gene multiplications that cause autosomal dominant PD. A sizeable body of work in neuronal systems implicates oxidative stress, mitochondrial dysfunction, defective protein degradation and the dysregulation of metal ions, in particular Mn2+, Fe2+, and Cu2+, in the pathogenesis of synucleinopathies.

Yeast models of synucleinopathy are created by expressing different forms of human α-Syn in yeast[35-40]. In one model, α-syn (WT or mutant) linked to green fluorescent protein (GFP) is expressed from a regulatable promoter at different expression levels (Box 1). GFP does not alter the biology or toxicity of the protein in yeast, thus providing a powerful tool for studying changes in α-syn distribution in living cells. In the yeast model, α-syn-GFP localizes to the plasma membrane, consistent with its known affinity with phospholipids. Yeast has constitutive vesicular secretion, so this is the expected localization of a protein that localizes

to synaptic vesicles in neurons. Consistent with a toxic gain-of-function, doubling the expression levels of α-syn dramatically changes its localization, leading to intracytoplasmic α-syn inclusions (Box 2) and cytotoxicity (growth inhibition and cell death). Key aspects of mammalian cellular pathology are recapitulated including defective vesicle trafficking and proteasomal degradation, reactive oxygen species, mitochondrial pathology and lipid droplet accumulation[37,40-42]. Cell death is apoptosis-like, indicated by loss of mitochondrial membrane asymmetry due to the externalization of phosphatidylserine and the release of cytochrome c from mitochondria.[37] Microarray analysis at different times after α-syn induction implicated early mitochondrial dysfunction and ER stress, followed by vesicle trafficking and sterol biosynthesis[42]. An analysis using conditional *SEC* mutants revealed α-syn is trafficked to the plasma membrane via the classical secretory pathway[36] .

Genetic and small molecule analysis– important themes and validation in neuronal systems. As noted above, the "humanized" yeast models for polyglutamine diseases and synucleinopathies recapitulate key features of the cellular pathology identified in the disease, providing the rationale for engaging the powerful analytic tools available in this system for molecular dissection of the cellular toxicity, and for high-throughput screens. In Boxes 1 and 2 we summarize a subset of published genetic and small molecule screen data from these yeast models. Modifiers of toxicity for different diseases are largely distinct, strongly suggesting that specific toxicity is dependent on the identity and nature of the protein being over-expressed. For example, contributing to polyQ toxicity are the unfolded protein response, impaired ERAD, oxidative stress and the kynurenine pathway, whereas contributors to

synuclein toxicity include blockage in ER-to-Golgi trafficking and vesicular transport, mitochondrial dysfunction and manganese sensitivity. Interestingly, the heat shock response was a pathway implicated by genetic modifiers for both models. Small molecule screens are identifying compounds that can reverse cellular defects in these models. For example, Box 2 details compounds identified in a recent screen of ~115,000 compounds and ~5 million cyclic peptides that rescue cellular toxicity in a synucleinopathy model[42, 67]. Importantly, the genetic and small molecule hits from genome-wide screening in yeast are being validated in neurons, both in culture and *in vivo* in higher organisms including worms, flies and rodents, firmly establishing *S. cerevisiae* as a tool for drug discovery in neurodegeneration. This is exemplified by the compound C2-8, identified in a yeast screen for small molecules that reduce polyglutamine aggregation. C2-8 has recently been shown to ameliorate neurodegeneration and motor deficits in a mouse model of HD[43]. Below, we offer select examples to illustrate ways in which the yeast model system has enriched our understanding of these diseases.

Insights into neuronal subtype vulnerability. Despite sharing a common mechanism intimately related to polyQ expansion and protein misfolding, polyQ diseases are a heterogeneous group, each affecting a distinct group of neurons and leading to distinct clinical phenotypes. With no clear relationship of expression levels to patterns of neuronal vulnerability or protein aggregation, these diseases exemplify the differential vulnerability of neuronal populations that is a pervading and extremely perplexing feature of neurodegenerative diseases. It may seem unlikely that expression of a polyQ-expanded protein in a unicellular organism could give clues to differential neuronal vulnerability in a

complex neurodegenerative disease. However, the yeast system has done just that. PolyQ toxicity in this model is critically dependent upon even subtle changes in the cellular proteome. Changes in the expression of just one other glutamine-rich protein can shift Htt-Ex1 from deadly to benign and *vice versa*. In some cases this seems to be due to the polyQ-expanded protein trapping and thereby titrating out an essential glutamine-rich protein[44]. In other cases, interaction with other non-essential glutamine-rich proteins can influence the conformational state of the Htt fragment[45]. A recent genome-wide suppressor screen supported this idea by showing that deletions of a number of glutamine/asparagine-rich proteins suppressed toxicity[32]. In experiments that would have been implausible in any other system, the yeast model also revealed an exquisite dependence of polyQ toxicity not only on the expression levels of other glutamine-containing proteins, but also on their conformation. For example, the glutamine-rich yeast protein Rnq1 exists in two different states, one soluble and the other a self-perpetuating amyloid (yeast prion). Strikingly, only when Rnq1 is in the prion state does the polyQ-expanded Htt-Ex1 fragment become toxic[30]. The human proteome contains a large number of glutamine- and asparagine-rich proteins. Some of these might have a capacity to change their conformational status in a similar way. It seems highly likely that differential neuronal vulnerability will be strongly influenced by changes in the expression and conformation of such proteins.

Studies in yeast have also revealed that the effect of the polyQ expansion is highly sensitive to flanking sequences. A recent study of 14 separate Htt-Ex1 constructs with varying sequences flanking the polyQ tract demonstrated that a proline-rich region adjacent to the polyQ tract in the normal human Htt protein is strongly protective, and that removing it can transform a protein from benign to toxic. As the disease-causing polyQ expansion

proteins differ profoundly in the amino acids flanking the polyQ region, the yeast data suggest that these flanking sequences are an important determinant of the unique toxicity and disease phenotype caused by each protein, whether by modulating protein folding or by altering protein-protein interactions. Interestingly, the effect also works in trans because the enhanced toxicity conferred on polyQ-expanded HttEx1 by the expression of other glutamine-rich proteins in the cell can be abrogated by the proline-rich region in Htt[46]. Such findings highlight the insight that can be gained from yeast into the cell-specific biology that contributes to differential neuronal vulnerability in these diseases.

Linking environmental and genetic causes. A number of environmental and genetic factors have been implicated in the pathogenesis of PD. It is unclear whether there is convergence upon a parkinsonian phenotype as a final common pathway, or whether these factors are mechanistically related. The yeast toolbox provides an unparalleled system to examine the cellular effects of genetic and environmental perturbations, and to determine whether and how they may be related. It is particularly compelling when disparate factors are causally linked by unbiased genetic analysis. For example, in a recent over-expression screen of the yeast synucleinopathy model that included ~60 metal transporters, only 3 were recovered as toxicity modifiers and two of these were known to be involved in the transport of manganese[47]. The first transporter, Pmr1p, transports Mn^{2+} and Ca^{2+} ions from the cytoplasm into the Golgi, and was an enhancer. The second transporter, Ccc1p, sequesters Mn^{2+} and Fe^{2+} ions into the vacuole of yeast cells and was a suppressor. This is intriguing because exposure to inhaled forms of manganese by miners has long been associated with increased risk of developing a type of parkinsonism[48]. The yeast interaction data set thus suggested a pathophysiological link between manganese and α-syn that would not be immediately

apparent from the distinctly different neuropathology of manganese exposure and PD.

Remarkably, the third transporter identified in the screen, YOR291w, also modulated sensitivity to Mn^{2+}. YOR291w is a transmembrane ATPase that is a member of the metal transport family and it strongly suppressed α-syn toxicity[47]. The human homolog of this highly conserved protein, ATP13A2, is believed to couple the hydrolysis of ATP to the transport of cations across various cellular membranes. Importantly, mutations in *ATP13A2*, also known as *PARK9* (Box2), were recently shown to cause early onset PD (Kufor-Rakeb syndrome)[49]. Beyond its utility as a drug discovery tool, this finding underscores the ability of the yeast model to identify a causal relationship between two previously unrelated proteins implicated in PD, allowing for detailed mechanistic investigation of its basis. Recent data demonstrates that the deletion of *YOR291w* (now also known as *YPK9* for "yeast *PARK9*") confers sensitivity of yeast to cadmium, nickel and selenium, in addition to manganese, reinforcing the relationship of PD to heavy metal ion transport and exposure[50]. The unique tractability of the yeast system will allow for further investigation of gene-environment interactions in PD and other synucleinopathies.

ResponseNet, creating disease networks. The extraordinary amount of molecular interaction data now identified in yeast places this model system at the forefront of analyzing a biological process at a systems level. Cellular responses to stimuli have most commonly been assessed with transcriptional (mRNA) profiling and genetic screens. Earlier suggestions of relatively little overlap between these two types of high-throughput analysis was recently confirmed in yeast by comparing the transcriptional gene expression and genetic modifier data for 179 distinct perturbations[51]. Important known components of well-studied pathways such as the DNA damage response were not identified by either high-throughput assay,

indicating that each method assessed different aspects of cellular response. A computational method called "ResponseNet" was devised to bridge gene expression and gene modifier data sets by utilizing a yeast interactome dataset relating 5,622 interacting proteins and 5,510 regulated genes via 57,955 protein-protein and protein-DNA interactions (Fig. 2)[51]. The ResponseNet flow algorithm was designed to identify the most probable pathways connecting genes and proteins, thus avoiding an unmanageable "hairball" of interactions. As proof-of-principle, the ResponseNet network succeeded in identifying components of well-studied pathways that had been known from painstaking individual analyses in the past but that had eluded both high-throughput genetic modifier and transcriptional datasets.

The ResponseNet algorithm was used to bridge high-throughput α-syn over-expression genetic modifier and microarray mRNA profile data sets. ResponseNet identified connections between pathways not prominently represented in either dataset but that had been previously associated with synucleinopathies, including ubiquitin-dependent protein degradation, nitrosative stress, cell-cycle regulation and vesicle-trafficking pathways. The heat shock response had previously been identified as a modulator of α-syn toxicity in a candidate-based genetic approach (Box 2)[37]. Interestingly, ResponseNet predicted the involvement of two highly conserved heat-shock regulators, the chaperone Hsp90 and the heat-shock transcription factor Hsf1 and identified a new regulator of the heat-shock response, all of which had been absent from the list of genetic modifiers used for the analysis. This finding corroborated earlier candidate-based analysis in yeast that implicated the heat shock response in α-syn toxicity[37]. ResponseNet predicted the importance of the mevalonate-ergosterol biosynthesis pathway in the response to α-syn. This pathway is targeted by the cholesterol-lowering statin drugs and synthesizes sterols as well as other

products with connections to α-syn toxicity, such as farnesyl groups required for vesicle trafficking proteins and ubiquinone, which is required for mitochondrial respiration. Statins and lipoprotein levels have been controversially linked to PD by a number of epidemiologic studies[52]. Lovastatin enhanced α-syn toxicity in the yeast model. ResponseNet thus provided new avenues to mechanistically explore the basis for previously identified connections in human epidemiology studies, as well as to discover new gene-environment relationships. We envisage the ResponseNet algorithm and other similar tools being developed in yeast will be invaluable in understanding the relationships between diverse genetic and environmental perturbations.

Concluding paragraph

To many, the idea of modelling neurodegenerative diseases in an organism without a nervous system, and a unicellular organism at that, may seem nothing short of absurd. And there are certainly many important aspects of these diseases that lie beyond the reach of *S. cerevisiae*. However, with highly conserved cellular processes, yeast provides a living test-tube in which to explore the fundamental derangements of cell biology that accompany and drive neurodegeneration. Yeast models faithfully recapitulate salient cellular and molecular pathologies associated with neurodegenerative diseases, providing the rationale for utilizing the unparalleled analytic tools available in this system for detailed molecular analysis. High-throughput genetic and small molecule screens in this system have already uncovered novel disease mechanisms, revealed connections between previously unrelated genetic and environmental susceptibilities, and provided new targets for therapeutic intervention. Key hits have been validated in animal and mammalian cell culture models of these diseases,

supporting the relevance of this approach. Finally, novel computational methods, coupled with knowledge of the yeast interactome, are providing a systems-based context in which to interpret large genomic and proteomic datasets generated not only in yeast, but also in other model systems and by human genome-wide analyses.

While all these achievements have surpassed initial expectations, the diminutive *S. cerevisiae* is certainly no stranger to playing pivotal roles. But beyond brewing of beer and leavening of bread, we suggest the wonders of modern molecular biology have now set the stage for yeast to transform our understanding of complex human disease processes including neurodegeneration.

Acknowledgments: We would like to thank members of the Lindquist laboratory for their insightful comments on the manuscript. SLL is an Investigator of the Howard Hughes Medical Institute. This work was supported by an HHMI Collaborative Innovation Award, NIH Udall program grant NS038372, and the Whitehead Institute Regenerative Biology Initiative.

BOX1

A yeast model of Huntington's disease and other polyglutamine expansion disorders

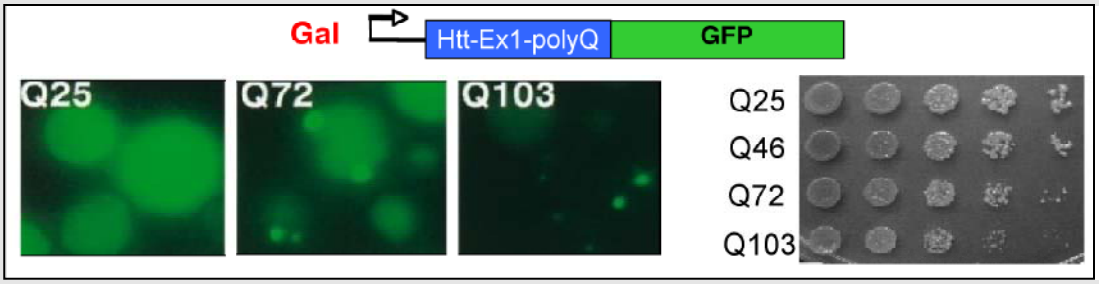


Figure courtesy of Martin Duennwald

GFP-tagged exon 1 of Huntingtin is expressed under a regulatable promoter with different polyglutamine (polyQ) tract lengths. Immunofluorescence (left panel) shows the intracytoplasmic aggregates form with higher tract lengths. A spot assay (serial dilution; right panel) shows decreased growth with higher tract lengths.

Genetic modifiers

- ***Candidate testing:*** suppressors included chaperones and protein remodelling factors such as, TRiC, Hsp104, and small heat shock proteins, and the essential ERAD proteins Npl4 and Ufd1, demonstrating a causal relationship between ERAD dysfunction and Htt-induced toxicity in this model[34].
- ***Gene-deletion genome-wide suppressor screen (Htt-ex1-103Q)*:** Suppressors were identified with roles in vesicle transport, vacuolar degradation, transcription and a large number of containing glutamine/asparagine-rich regions, some of which were yeast prions. **Bna4 (kynurenine 3-monooxygenase; KMO)**, a mitochondrial enzyme in the kynurenine pathway of tryptophan degradation, was a potent suppressor shown to be essential in reactive oxygen species (ROS) generation in the yeast model. This study demonstrated that Htt-Ex1 aggregation occurs in several yeast gene deletion strains that suppress toxicity, disconnecting toxicity from the formation of large aggregates[32].
- ***Synthetic lethality enhancer screen (Htt-ex1-53Q):*** Modifier classes included proteins involved in response to stress (including some proteins required for redox/oxidative stress response in yeast such as glutathione synthase), protein folding, and ubiquitin-dependent protein catabolism.[53]

Small molecule screening

- Utilized aggregation of polyQ-GFP as a screenable phenotype (even if large visible 'aggregates' are not the toxic species, the formation of macroaggregates is probably a useful surrogate for upstream events that are critical to toxicity).
- **16000 compound library screen** yielded a potent inhibitor of aggregation, C2-8 [54].
- A screen for ~5000 natural substances revealed that the green tea polyphenol (-)-epigallocatechin-3-gallate (EGCG) potently inhibited the aggregation of polyQ-expanded Htt-Ex1 in a dose-dependent manner and this was verified in yeast and higher model systems [40].

Validation in higher model systems.

- **Chaperones Hsp70, Hsp40, and TRiC, and the protein remodelling factor Hsp104** reduced the aggregation of Htt-Ex1 fragments *in vitro*, and prolong survival in fly, mouse and mammalian cell models of HD[31, 55-57].
- **ER stress** was shown to be an early presymptomatic event persisting throughout their lifespan in an HD mouse model, and in postmortem brain tissue of HD patients[58], validating the finding in yeast of polyQ-dependent ER stress and ERAD inhibition.
- **Dysfunction of the kynurenine pathway** was linked to oxidative stress in animal models of HD and in HD[59], validating the finding that toxicity and ROS generation in the yeast HD model is dependent on the kynurenine 3-monooxygenase homolog Bna4.[32]
- The clear disconnection in yeast screens between genetic modifiers of toxicity and the extent of Htt–Ex1 aggregation, implying large aggregates are themselves non-pathogenic, has been validated in multiple model systems including primary neuronal culture and higher organisms[60, 61].
- **Potential therapeutic compounds** identified in yeast are being validated in higher model systems. **C2-8** inhibited aggregation of mutant Htt in neurons within a hippocampal slice culture from a mouse HD model, ameliorated neurodegeneration in a *Drosophila* HD model and rescued motor deficits and neurodegeneration in a HD mouse model [43]. Interestingly, C2-8 is a structural analog of the compound Ro 61-8048, a high-affinity small-molecule inhibitor of KMO, underscoring the genetic data linking this pathway to toxicity. **EGCG** rescued photoreceptor degeneration and motor function in HD transgenic flies overexpressing polyQ-expanded Htt-Ex1[62].

BOX2

A yeast model of Parkinson's disease (PD) and other synucleinopathies

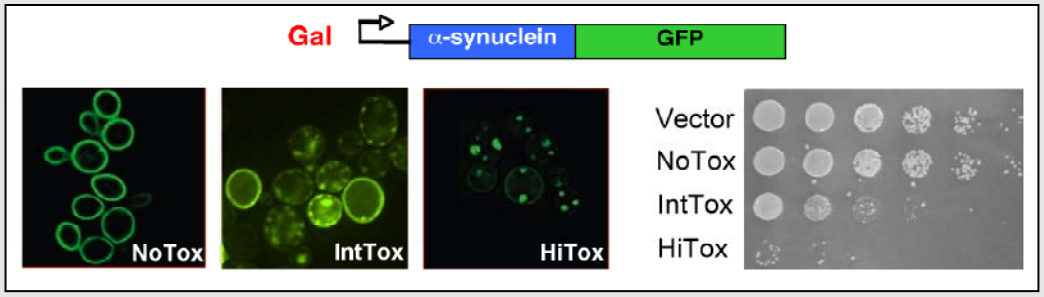


Figure courtesy of Julie Valastyan

α-Syn-GFP is expressed under a regulatable promoter at different levels of expression resulting in no toxicity (NoTox), intermediate toxicity (IntTox) and high toxicity (HiTox). Immunofluorescence (left panel) shows the localization shifts from the plasma membrane to intracytoplasmic aggregates with increasing expression. A spot assay (serial dilution; right panel) shows decreased growth with higher levels of α-Syn expression.

Genetic modifiers

- ***Over-expression screen (intermediate toxicity wild-type α-Syn strain)***: 77 modfiers prominently represented vesicle trafficking, metal ion transport, osmolyte synthesis, protein phosphorylation, nitrosative stress and trehalose metabolism. One strong suppressor was the yeast homolog of *ATP13A2*/*PARK9* (see discussion in text)[13, 47].
- ***ER-to-Golgi trafficking***: Suppressors promoted and enhancers inhibited ER-to-Golgi anterograde transport – foe example, Ypt1 (the homolog of mammalian *RAB1*), a GTPase that promotes the movement of vesicles from ER to Golgi, suppressed toxicity; *GYP8*, the GTPase activating protein that converts Rab1 from its active GTP-bound state to its inactive GDP bound state, enhanced toxicity. The modifier set suggested α-Syn is likely to be inhibiting the docking or fusion of vesicles to the Golgi, subsequently substantiated by ultrastructural studies and analysis in a cell-free system. Ultrastructural analysis revealed the large cytoplasmic α-Syn inclusions seen with immunofluorescence are accumulations of undocked vesicles associated with α-Syn [63, 64]. Biochemical studies have showed that α-Syn expression induces ER stress by specifically blocking the degradation of misfolded ER proteins that require transport from the ER to the Golgi prior to degradation[13, 47, 63].
- ***Trehalose* pathway**: 3 genes involved in trehalose biosynthesis and metabolism suppressed α-Syn toxicity. Trehalose is a chemical chaperone found in yeast that promotes correct protein folding, inhibits aggregation, and allows organisms, even in which it is not endogenously synthesized, to survive extreme conditions of environmental stress. Trehalose and other osmolytes are potentially therapeutic modulators of proteotoxic stress because they can cross the blood-brain barrier and are generally nontoxic.
- ***Candidate testing***: Heat shock and heat shock response genes were potent suppressors of α-Syn toxicity, and apoptotic cell death was found to be dependent upon the yeast metacaspase Yca1[37]. The ResponseNet analysis (see text for details) identified the genetic screen hit Gip2 as a novel conserved regulator of the heat shock response (see text for details)[51].
- ***Over-expression screen (mutant A30P α-Syn)***: Ypp1 was a suppressor, antagonizing ROS accumulation and toxicity when over-expressed and binding to A30P α-Syn and directing it towards degradation in the vacuole[65].
- ***Synthetic lethality enhancer screen*:** 32% of hits related to lipid metabolism and vesicular transport[53].

Small molecule screening

- ***~10,000 compound library screen***: This screen identified flavonoids (including EGCG and quercetin) as suppressors of α-Syn toxicity at micromolar concentrations, with SAR analysis implicating reduction in oxidative stress and metal chelation as possible mechanisms of action[66].
- ***~115,000 compound library screen (commercial, natural product, NCI libraries):*** 4 structurally related compounds were found to antagonize α-Syn-mediated inclusion formation and α-Syn toxicity at low micromolar concentrations, rescuing the ER-to-Golgi vesicular trafficking and mitochondrial defects, and greatly reducing the formation of α-Syn inclusions[42]. These four compounds did not rescue growth of yeast cells expressing polyQ-expanded Htt-Ex1, arguing for specificity.
- ***Cyclic peptide screen***: From a pool of 5 million transformants, two related cyclic peptide constructs specifically reduced the toxicity of human α-Syn; these peptides were as efficacious as the leading suppressors from genetic screens although they appear to function downstream of the ER-to-Golgi trafficking defect; structure-activity relationship (SAR) data was rapidly generated for the peptides using point mutagenesis[67].

Validation in higher model systems

- ***Impaired vesicle trafficking***: Mice lacking α-Syn gene have reduced pools of synaptic vesicles and exhibit an enhancement in the activity-dependent release of dopamine; conversely neurons over-expressing α-Syn exhibit a reduction in stimulation-dependent neurotransmitter release due to a reduction in vesicular fusion with the plasma membrane[68].
- ***Validation of key genetic modifiers***: The human homolog of Rab1/Ypt1 suppressed α-Syn toxicity in DA neurons of fruit flies, nematodes and in mixed primary rat neuronal cultures[13]. In addition, *RAB3A*, which is highly expressed in neurons and localized to presynaptic termini, and *RAB8*A, which is localized to post-Golgi vesicles, suppressed toxicity in neuronal models of PD[63].
- ***Validation of small molecules***: The two most potent compounds from the small molecule screen and the two cyclic peptides identified as suppressors in the yeast synucleinopathy model were also suppressors in the nematode and rat neuronal synucleinopathy models[42, 67]. The two leading compounds from the small molecule screen also rescued rat DA neurons from toxicity induced by the mitochondrial toxin rotenone, strengthening the connection between α-Syn and mitochondrial toxicity.

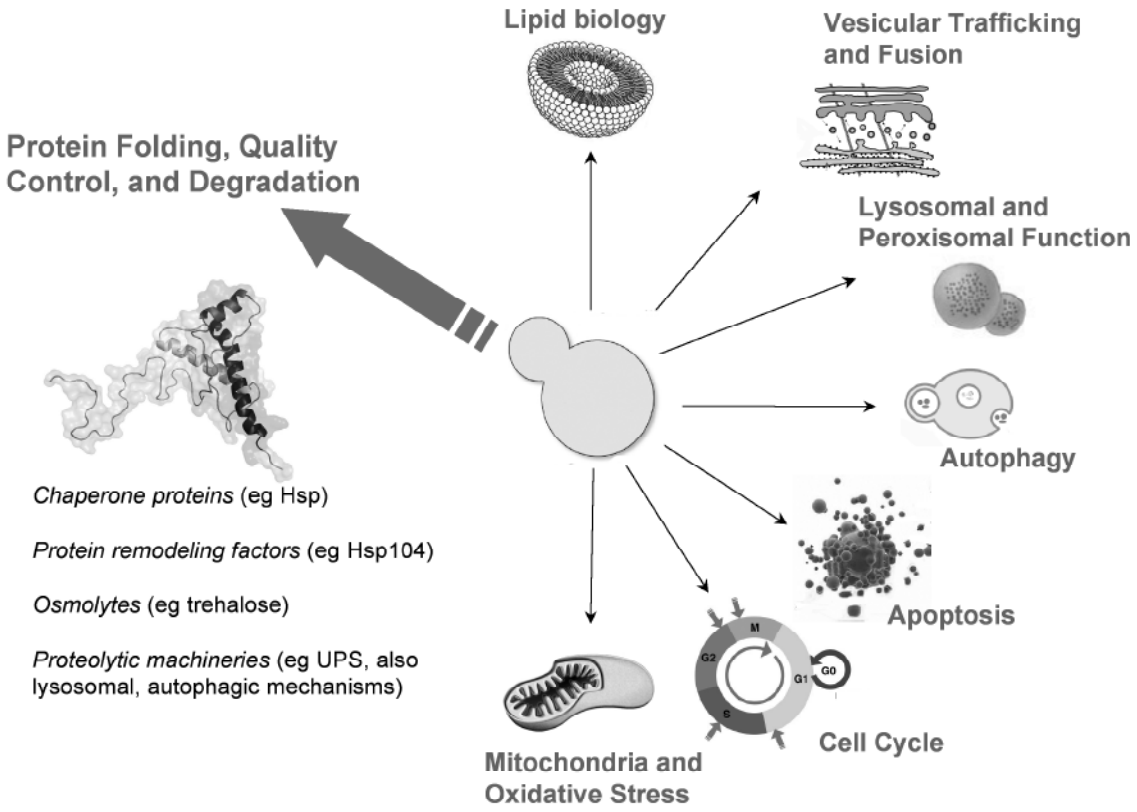


Figure 1: Conserved cellular biology in yeast. Numerous cellular pathways of high relevance to neurodegeneration are well conserved in yeast. Indeed, many owe their characterization to molecular dissection in the yeast model system (see text for details). While there are undoubtedly conserved apoptotic mechanisms in yeast, the presence of a classical programmed cell death pathway remains controversial. Given the fundamental role played in neurodegenerative diseases by protein misfolding, conservation of protein folding, quality control and degradation machineries is particularly pertinent. This machinery includes: 1) **Chaperone proteins** (for example heat shock proteins Hsp40, Hsp60, Hsp70, Hsp90) and their cofactors: bind to specific protein folding intermediates, maintain their soluble state and promote their maturation; 2) **Protein remodelling factors** (such as Hsp104 in yeast, the ortholog of mammalian p97): 3) **Osmolytes** (such trehalose, betaine and triethylamine-N-oxide) serve as "chemical chaperones" by changing the hydrodynamic interactions between proteins and promoting maintenance of the folded state.interact with improperly folded proteins and re-arrange them using ATP; 4) **Proteolytic machineries** selectively degrade unstable and misfolded proteins and include the ubiquitin-proteasome system, chaperone-mediated autophagy, and autophagy lysosomal system; 5) **Membrane-bound compartments** (including the nuclear envelope, mitochondria, Golgi apparatus, ER, lyososomes and endosomes) segregate distinct functionally related proteins; 6) **Vesicular trafficking machinery** (including SNARE, clathrin/adaptin, COPI and COPII, ESCRT complexes): traffics proteins between certain membrane-bound compartments, including the ER to Golgi and ER to lysosomes, often via transport vesicles. Abbreviations: ER endoplasmic reticulum; SNARE soluble N-ethylmaleimide-sensitive factor attachment protein receptor; ESCRT endosomal sorting complex required for transport; UPS ubiquitin-proteasome system.

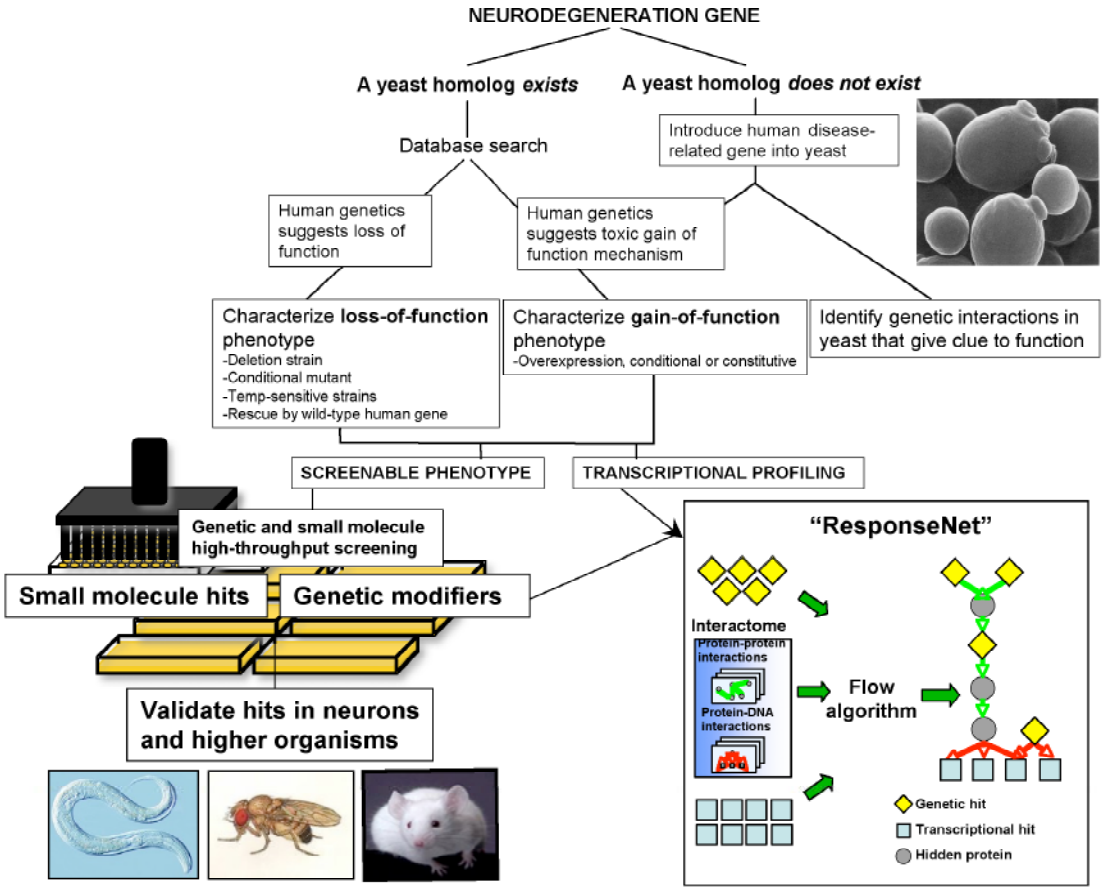


Figure 2: Creating, characterizing and screening a yeast model for neurodegenerative disease. When a neurodegenerative disease-causing mutation is identified, the first question to ask is whether a homolog exists in yeast or not by consulting existing yeast databases (see Table 2). If a yeast homolog exists, there may be substantial characterization of the gene in question. Loss-of-function genes can be characterized by gene deletion, conditional mutants. Complementation is established when a human gene can "rescue" the loss-of-function phenotype in yeast. If a homolog does not exist, the human gene product can be introduced into yeast. This can either be purely to analyze its function by harnessing the yeast toolbox (genetic and protein interactions) or, in cases where human genetics suggests a toxic gain-of-function mechanism, over-epxression of the gene in yeast may recapitulate cellular pathology associated with the disease. Once a yeast model is created by either gene disruption or over-expression, analysis proceeds along the two paths: 1) profiling – most commonly transcriptional; 2) screening against small molecule and genetic libraries. A recently developed algorithm called ResponseNet makes use of the yeast interactome and can be used to bridge transcriptional and genetic modifier data sets, uncovering "hidden proteins" in a global picture of cellular response (see text for more details). Genetic modifiers and small molecule hits from yeast screens are validated in cultured or whole organism neuronal systems.

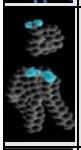
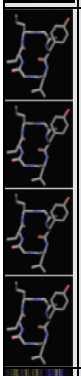
	**1) *FUNCTIONAL GENOMICS:* GENETIC SCREENING LIBRARIES** **Systematic large-scale profiling of genetic interactions within a cell.** **Deletion mutant collection:** A collection of ~6000 heterozygous diploid strains, each deleted for a specific gene. In practice, a collection of 4000 haploid strains each deleted for a nonessential gene have been used for most screens. **Essential gene mutant collection**: A collection in which essential genes are placed under the regulation of a repressible promoter. **Hypomorphic allele collections**: Reduced function strains (e.g. conditional mutants or gene manipulations to lower transcript levels). **Comprehensive gene over-expression libaries**: The complete set of yeast ORFs has been cloned into various vectors that allow expression of an individual tagged ORF constitutively or under the control of an inducible promoter. The FLEXgene collection contains 5000 sequence-verified ORFs. **Genomic plasmid libraries**: Libraries encompassing both the protein-encoding and non-encoding genome, offering the advantage of containing genes under the control of their endogenous promoter[69].
	**2) *TRANSCRIPTOMICS*:** **Systematic large-scale profiling of the expression level of mRNAs in a given cell population** *S. cerevesiae* was the first organism for which cDNA microarrays were used for genome-wide transcriptional profiling [70], introducing a powerful method to generate insight into the biological effects of genetic, chemical and environmental manipulation. Profiles now exist for thousands of manipulations.
	**3) *PROTEOMICS*** **Systematic large-scale profiling of the proteins of a cell.** **Yeast two-hybrid interaction screen:** A protein of interest is fused to the DNA-binding domain of the transcriptional activator GAL4, and screened against a library consisting of strains expressing every yeast protein fused to the GAL4 activation domain. If the two proteins interact, GAL4 activity is reconstituted and a selectable marker is transcribed[1] **Co-immunoprecipitation and localization studies:** Numerous fusions (HA, TAP, GFP, EGFP, ECFP, EYFP, Cerulean, DsRed, GST) for protein localization and for the recovery and analysis of individual proteins and protein complexes, recently incorporated into a collection of vectors into which a yeast ORF can be readily introduced[71, 72]. **Protein microarray:** Functional arrays can be used to analyze the biochemical activities of deposited proteins. Nickel or nitrocellulose-coated slides containing tagged proteins can be used to analyze protein-protein, protein-lipid and protein-nucleic acid interactions[1].
	**4) *METABOLOMICS*** **Systematic large-scale profiling of the small molecule metabolites of a cell.** **Lipidomics:** The quantitation of ~the entire set of yeast lipids and lipid-related metabolites (the "lipidome"), providing unprecedented power to study a class of molecules that is becomingly increasingly recognized as crucial in complex cellular processes including signaling and vesicle trafficking[73, 74].
	**5) *CHEMICAL GENOMICS AND CHEMICAL GENETICS*** **Systematic large-scale profiling of the biological effects of libraries of small molecules (chemical genomics) and their interaction with genetic perturbations (chemical genetics).** **Small molecule screens:** Cost effectiveness and short generation time allow yeast to be plated in 96, 384- and 1536-well format for automated robotic screening of a phenotype or process against a small molecule library. **Chemical genetic profiling**: A compound can be screened against the entire library of haploid deletion strains (generating a "fitness profile") to help identify the pathway and potentially the actual protein targeted by the compound. Comparing the compound's genetic profile with synthetic genetic lethal interactions enhances the power of the method[75, 76]. **Chemical genetic mapping**: More than 1000 chemical and environmental perturbations were recently tested on every strain of the nonessential deletion library, creating an extensive chemical genetic analysis of *S. cervesiae,* providing an extraordinary resource for predicting drug targets and drug synergy[77]. **Cyclic peptide libraries**: Cyclic peptides are small proteins with a ring architecture. Recently, libraries of millions of cyclic peptides *in vivo* have been generated, providing a largely unexplored chemical space for screening and a system in which structure-activity relationships (SAR) can be rapidly generated for the peptides using point mutagenesis[67].
	**6) *PHENOMICS:* PHENOTYPE MICROARRAY ANALYSIS** A two-dimensional array technology that uses cellular respiration as a universal reporter to measure the "phenome" of a genetic or chemical perturbation. Thousands of phenotypes are measured simultaneously including: tests of C, N, P and S catabolism; osmotic, pH and chemical sensitivity; small molecule and macromolecule biosynthesis; cell surface structure and transport[78].

Table 1: "Yeastomics": Modern tools available in *S. cerevesiae* that enable systematic analysis of a cellular process or a phenotype

Database	Web URL
Saccharomyces Genome Database (SGD)	http://www.yeastgenome.org/
The Comprehensive Yeast Genome Database (from MIPS)	http://mips.gsf.de/genre/proj/yeast/index.jsp
Saccharomyces Genome Deletion Project	http://www-sequence.stanford.edu/group/ yeast_deletion_project/deletions3.html
European Saccharomyces Cerevisiae Archives for Functional analysis (Euroscarf)	http://web.uni-frankfurt.de/fb15/mikro/euroscarf/
Stanford Microarray database	http://genome-www5.stanford.edu/
General Repository for Interaction Datasets (BioGRID)	General Repository for Interaction Datasets (BioGRID) http://www.thebiogrid.org/
Yeast Global Microarray Viewer	http://www.transcriptome.ens.fr/ymgv/
Yeast Resource Center	http://depts.washington.edu/yeastrc/pages/overview.html
Yeast GFP Fusion Localization	Database http://yeastgfp.ucsf.edu/
Yeast FLEXgene collection	http://flex.med.harvard.edu/FLEX/
Information Hyperlinked over Proteins	http://www.ihop-net.org/UniPub/iHOP/
Database of Interacting Proteins (DIP)	http://dip.doe-mbi.ucla.edu/
S. cerevisiae Advanced Gateway Destination Vectors	http://www.addgene.org/yeast_gateway

Table 2: A selection of online yeast databases.

Disease Category	Human	Yeast	Disease	Human protein product / Inheritance / reference(s)
ATAXIA	*FRDA*	*YFH1*	FA	Frataxin. a nuclear-encoded mitochondrial protein involved in iron sulfur cluster enzyme biogenesis; autosomal recessive mostly caused by expansion of an unstable GAA repeat in the first intron; FA is the most common inherited ataxia[25].
ATAXIA	*ATXN-2*	*PBP1*	SCA2	Ataxin-2, putative role in RNA metabolism; autosomal dominant caused by an expanded polyglutamine tract.
ATAXIA	*CACNA1A*	*CCH1*	SCA6	Ataxin-6, α1A transmembrane subunit of the P/Q-type voltage gated calcium channel; autosomal dominant caused by an expanded polyglutamine tract.
ATAXIA	*SCA7*	*SGF73*	SCA7	Ataxin-7, a subunit of histone acetyltransferease complex; autosomal dominant caused by an expanded polyglutamine tract.
ATAXIA	*SCA10*	*ATXN10*	SCA10	Ataxin-10; autosomal dominant caused by a pentanucleotide (ATTCT) repeat expansion in the SCA10 gene.
ATAXIA	*TBP*	*SPT15*	SCA17	TATA-box binding protein; autosomal dominant SCA caused by an expanded polyglutamine tract.
ATAXIA	*TDP1*	*TDP1*	SCAN1	TDP1, a DNA repair protein; autosomal recessive.
ATAXIA	*ATM*	*TEL1*	AT	ATM, a key regulator of the DNA damage response; autosomal recessive cerebellar degeneration.
DEMENTIA	*SORL1/LR11*	*PEP1/VPS10* *VTH1* *VTH2* *YNR065C*	AD	Sortilin-1 (SORL1, LR11), a neuronal sorting receptor of the LDL receptor family; genetically associated with late-onset AD.
DEMENTIA	*CHMP2B*	*DID4*	FTD	CHMP2B, an ESCRT-III subunit, important in endosomal-lysosomal trafficking; autosomal recessive.
LYSOSOMAL STORAGE	*CTSD*	*PEP4*	NCL	Cathepsin D. a lysosomal protease; autosomal recessive congenital NCL.
LYSOSOMAL STORAGE	*CLN3*	*YHC3 (BTN1)*	NCL (BD)	CLN3. a lysosomal transmembrane protein; autosomal recessive juvenile NCL (also known as BD).[79]
LYSOSOMAL STORAGE	*SMPD1*	*PPN1*	NP type A	Sphingomyelinase, a lysozomal enzyme; autosomal recessive.
LYSOSOMAL STORAGE	*NPC1*	*NCR1*	NP type C	NPC1, a late endosomal protein involved in cholesterol trafficking; autosomal recessive; associated with intraneuronal accumulation of unesterified cholesterol[80].
MOTOR NEURON (UPPER/LOWER)	*SOD1*	*SOD1*	ALS	Superoxide dismutase, a major antioxidant enzyme; autosomal dominant.
MOTOR NEURON (UPPER/LOWER)	*VAPB*	*SCS2* *SCS22*	SMA/ALS	VAPB, a membrane protein involved in vesicle trafficking.
MOTOR NEURON (LOWER)	*DCN1*	*NIP100*	ALS	p150 subunit of dynactin, a protein involved in axonal transport; autosomal dominant (likely dominant negative mutation).[81]
MOTOR NEURON (UPPER)	*REEP1*	*YOP1*	HSP	REEP1, a mitochondrial protein; autosomal dominant.
MOTOR NEURON (UPPER)	*SPG7*	*AFG3* *RCA1*	HSP	Paraplegin, mAAA protease; autosomal recessive; leads to axonal degeneration of corticospinal tracts and dorsal columns[82].
MOVEMENT	*DJ-1*	YDR533Cp	PD	DJ-1, a redox-activated chaperone; autosomal recessive parkinsonism.
MOVEMENT	*PANK2*	*YDR531W*	PKAN	Pantothenate kinase, involved in CoA biosynthesis; autosomal recessive parkinsonism and brain iron accumulation (PKAN).
MOVEMENT	*UCHL1*	*YUH1*	?PD	UCHL1, protein of unclear function; controversial susceptibility gene for familial PD; modulates toxicity in an AD mouse model.
MOVEMENT	*VPS13A*	*VPS13*	NA	Chorein, putative involvement in membrane protein trafficking; autosomal recessive movement disorder associated with acanthocytes in the blood and degeneration of the basal ganglia.
PERIPHERAL NEUROPATHY	*GARS*	*GRS1* *GRS2*	CMt2D Distal SMA-V	Glycyl-tRNA synthetase; autosomal dominant degeneration of peripheral sensory and motor neurons (CMT2D) or just motor neurons (distal SMA-V).
PERIPHERAL NEUROPATHY	*GDAP1*	*GTT2*	CMT4A	GDAP1 (ganglioside-induced differentiation-associated protein); autosomal recessive degenerative disease of peripheral motor and sensory neurons
PERIPHERAL NEUROPATHY	*MTMR2* *SBF2/MTMR13*	*YMR1*	CMT4B1 CMT4B2	MTMR2 and MTMR13 are myotubularin-related proteins linked with vesicular trafficking; autosomal recessive.
PERIPHERAL NEUROPATHY	*FIG4*	*FIG4*	CMT4J	FIG4, a phosphatase in the vacuolar membrane associated with endosome-lysosomal trafficking; autosomal recessive.
PERIPHERAL NEUROPATHY	*YARS*	*TYS1*	DICMT-C	Tyrosyl-tRNA synthetase; autosomal dominant degeneration of peripheral sensory and motor neurons.
PERIPHERAL NEUROPATHY	*SPTLC1*	*LCB1*	HSN	SPT, serine palmitoyltransferase involved in sphingolipid synthesis; autosomal dominant degeneration of peripheral sensory neuron.
WHITE MATTER	*ABCD1*	*PXA1* *PXA2*	X-linked ALD	ABCD1, a peroxisomal protein involved in very long chain fatty acid transport; X-linked ALD degenerative white matter disease[83].
WHITE MATTER	*PEX1* *PEX5* *PEX10* *PEX13* *PEX14* *PEX19*	*PEX1* *PEX5* *PEX10* *PEX13* *PEX14* *PEX19*	Zellweger syndrome	Peroxisome biogenesis proteins; autosomal recessive degenerative white matter disease.
WHITE MATTER	*XPC* *ERCC2* *DDB1* *DDB2* *RAD1* *RAD30*	*RAD4* *RAD3* *RSE1* *YDL156W* *ERCC4* *POLH*	XP type C XP type D XP type E XP type E XP type F XP variant	Multiple DNA repair proteins; autosomal recessive; neurologic manifestations, when present, generally include one or more of dementia, ataxia, peripheral neuropathy, deafness.

Table 3: A partial list of neurodegenerative disease genes with *S. cerevesiae* homologs. Many genes with less sequence homology not listed here may still have highly conserved domain homology and function. Shaded rows depict genes that have been used to create yeast models. See references for more details. Abbreviations AD Alzheimer's disease; ALD adrenoleukodystrophy; ALS amyotrophic lateral sclerosis; AT ataxia telangiectasia; ATM ataxia telangiectasia mutated; BD Batten disease; CMT Charcot-Martie-Tooth disease; DICMT dominant intermediate Charcot-Marie-Tooth; FA Friedreich's ataxia; FTD frontotemporal dementia; GDAP1 ganglioside-induced differentiation-associated protein 1; HSN hereditary sensory neuropathy; HSP hereditary spastic paraplegia; NA neuroacanthocytosis; NCL neuronal ceroid lipofuscinosis; NP Niemann-Pick disease; PD Parkinson's disease; PKAN pantothenate kinase-associated neurodegeneration; REEP1 receptor expression enhancing protein; SCA spinocerebellar ataxia; SCAN spinocerebellar ataxia with axonal neuropathy; SBF2 SET binding factor-2; SMA spinall muscular atrophy; TDP1 Tyrosyl-DNA phosphodiesterase 1; UCHL1 ubiquitin carboxyl-terminal esterase-L1; VAPB vesicle-associated membrane protein-B; XP xeroderma pigmentosum.

Disease	Aggregating protein	Yeast model	Ref
AD	beta-amyloid (Aβ; cleaved from APP through the action of two enzymes, β- and γ-secretase)	1) APP co-expression with reconstituted β- and γ-secretase. *Phenotype*: Cleavage products of APP produced, allowing monitoring of protease activity and identification of small molecule inhibitors of β-secretase.	84
		2) Aβ expressed as a fusion protein with the yeast prion Sup35p. *Phenotype*: Sup35p reporter gene function serves as a surrogate for Aβ aggregation.	85, 86
		3) Aβ expressed as a fusion protein with GFP. *Phenotype*: Inhibition of growth and heat shock response.	87
		4) Exogenous Aβ applied to yeast, fibrillar compared to non-fibrillar/oligomeric. *Phenotype*: Non-fibrillar/oligomeric Aβ caused enhanced loss of cell viability compared to fibrillar Aβ.	88
Synucleinopathies, including PD	α-Synuclein	Expression of α-Synuclein *Phenotype*: Cytosolic aggregation of a-synuclein associated with vesicular membrane; oxidative stress, lipid droplet accumulation, vesicle trafficking defects, and mitochondrial perturbations.	35-40
Polyglutamine expansion diseases, including HD	Many proteins with polyglutamine expansions, including Huntingtin	Expression of huntingtin exon 1 with a polyglutamine tract of various lengths. *Phenotype*: Cytoplasmic aggregation, ERAD defects and proteasome impairment with expanded tracts (eg 103Q).	28-31
FTD-U and ALS	TDP-43	Expression of TDP-43 fused to GFP. *Phenotype*: Increased expression leads to redistribution from nucleus to cytoplasm with concomitant aggregation and inhibition of growth.	89
Prion disease	PrP	Expression of PrP with or without a signal sequence or expression of PrP fused to yeast prions. *Phenotype*: Cytosolic PrP forms insoluble PrP^{SC}, and is capable of self-propagation; growth impairment occurs in ERAD or UPR-deficient yeast.	90-93
Tauopathy including AD, FTD-tau, PSP, CBD.	Tau	Expression of human tau (3 repeat and 4 repeat isoforms of wild-type tau, P301L mutant tau). *Phenotype*: Increased tau phosphorylation and aggregation, the latter enhanced by oxidative stress and mitochondrial dysfunction; decreased longevity.	40

Table 4: Neurodegenerative diseases putatively caused by toxic gain of protein function mechanisms, associated with intraneuronal and intraglial aggregates of proteins and modelled in yeast by overexpression of these proteins. Abbreviations: AD Alzheimer's disease; APP amyloid precursor protein; CBD corticobasal degeneration; ERAD endoplasmic reticulum-associated protein degradation; FTD-tau frontotemporal dementia associated with tau-immunoreactive inclusions; FTD-U frontotemporal dementia associated with ubiquitin-immunoreactive tau-immunonegative inclusions; HD Huntington's disease; PD Parkinson's disease; PSP progressive supranuclear palsy; UPR unfolded protein response.

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1. Smith, M.G. & Snyder, M. Yeast as a model for human disease. *Current protocols in human genetics / editorial board, Jonathan L Haines [et al]* **Chapter 15**, Unit 15.6 (2006).
2. Brandis, K.A. et al. alpha-Synuclein fission yeast model: concentration-dependent aggregation without plasma membrane localization or toxicity. *J Mol Neurosci* **28**, 179-91 (2006).
3. Codlin, S. & Mole, S.E. S. pombe btn1, the orthologue of the Batten disease gene CLN3, is required for vacuole protein sorting of Cpy1p and Golgi exit of Vps10p. *J Cell Sci* **122**, 1163-73 (2009).
4. yeastgenome.org.
5. Botstein, D., Chervitz, S.A. & Cherry, J.M. Yeast as a model organism. *Science* **277**, 1259-60 (1997).
6. Bassett, D.E., Boguski, M.S. & Hieter, P. Yeast genes and human disease. *Nature* **379**, 589-90 (1996).
7. Foury, F. Human genetic diseases: a cross-talk between man and yeast. *Gene* **195**, 1-10 (1997).
8. Lindquist, S.K., Liming, S. Investigating protein conformation-based inheritance and disease in yeast. *Philosophical Transactions of the Royal Society B: Biological Sciences* **356**, 169-176 (2001).
9. Tessier, P. & Lindquist, S. Unraveling infectious structures, strain variants and species barriers for the yeast prion [PSI+]. *Nat Struct Mol Biol* **16**, 598-605 (2009).
10. Knott, A.B., Perkins, G., Schwarzenbacher, R. & Bossy-Wetzel, E. Mitochondrial fragmentation in neurodegeneration. *Nat Rev Neurosci* **9**, 505-18 (2008).
11. Malhotra, V. & Emr, S.D. Rothman and Schekman SNAREd by Lasker for trafficking. *Cell* **111**, 1-3 (2002).
12. Littleton, J.T. & Bellen, H.J. Synaptotagmin controls and modulates synaptic-vesicle fusion in a Ca(2+)-dependent manner. *Trends Neurosci* **18**, 177-83 (1995).
13. Cooper, A.A. et al. Alpha-synuclein blocks ER-Golgi traffic and Rab1 rescues neuron loss in Parkinson's models. *Science* **313**, 324-8 (2006).
14. Lin, W. & Popko, B. Endoplasmic reticulum stress in disorders of myelinating cells. *Nat Neurosci* **12**, 379-85 (2009).
15. Scheper, W. & Hoozemans, J.J. Endoplasmic reticulum protein quality control in neurodegenerative disease: the good, the bad and the therapy. *Curr Med Chem* **16**, 615-26 (2009).
16. Herrup, K. & Yang, Y. Cell cycle regulation in the postmitotic neuron: oxymoron or new biology? *Nat Rev Neurosci* **8**, 368-78 (2007).
17. Jin, C. & Reed, J.C. Yeast and apoptosis. *Nat Rev Mol Cell Biol* **3**, 453-9 (2002).
18. Cheng, W.C., Leach, K.M. & Hardwick, J.M. Mitochondrial death pathways in yeast and mammalian cells. *Biochim Biophys Acta* **1783**, 1272-9 (2008).
19. Khoury, C. & Greenwood, M. The pleiotropic effects of heterologous Bax expression in yeast. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research* **1783**, 1449-1465 (2008).
20. Nakatogawa, H., Suzuki, K., Kamada, Y. & Ohsumi, Y. Dynamics and diversity in autophagy mechanisms: lessons from yeast. *Nat Rev Mol Cell Biol* **10**, 458-67 (2009).
21. Levine, B. & Kroemer, G. Autophagy in the pathogenesis of disease. *Cell* **132**, 27-42 (2008).
22. Botstein, D. & Fink, G.R. Yeast: an experimental organism for modern biology. *Science* **240**, 1439-43 (1988).
23. Winston, F. Yeast genetics.
24. van Anken, E. & Braakman, I. Endoplasmic reticulum stress and the making of a professional secretory cell. *Crit Rev Biochem Mol Biol* **40**, 269-83 (2005).
25. Knight, S., Kim, R., Pain, D. & Dancis, A. The Yeast Connection to Friedreich Ataxia. *The American Journal of Human Genetics* **64**, 365-371 (1999).
26. Orr, H.T. & Zoghbi, H.Y. Trinucleotide repeat disorders. *Annu Rev Neurosci* **30**, 575-621 (2007).
27. Davies, S.W. et al. Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD mutation. *Cell* **90**, 537-48 (1997).
28. Hughes, R.E. et al. Altered transcription in yeast expressing expanded polyglutamine. *Proc Natl Acad Sci USA* **98**, 13201-6 (2001).
29. Krobitsch, S. & Lindquist, S. 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**, 1589-94 (2000).
30. Meriin, A.B. et al. Huntington toxicity in yeast model depends on polyglutamine aggregation mediated by a prion-like protein Rnq1. *J Cell Biol* **157**, 997-1004 (2002).
31. Muchowski, P.J. et al. Hsp70 and hsp40 chaperones can inhibit self-assembly of polyglutamine proteins into amyloid-like fibrils. *Proc Natl Acad Sci USA* **97**, 7841-6 (2000).

32. Giorgini, F., Guidetti, P., Nguyen, Q., Bennett, S.C. & Muchowski, P.J. A genomic screen in yeast implicates kynurenine 3-monooxygenase as a therapeutic target for Huntington disease. *Nat Genet* **37**, 526-31 (2005).
33. Sokolov, S., Pozniakovsky, A., Bocharova, N., Knorre, D. & Severin, F. Expression of an expanded polyglutamine domain in yeast causes death with apoptotic markers. *Biochim Biophys Acta* **1757**, 660-6 (2006).
34. Duennwald, M.L. & Lindquist, S. Impaired ERAD and ER stress are early and specific events in polyglutamine toxicity. *Genes Dev* **22**, 3308-19 (2008).
35. Chen, Q., Thorpe, J. & Keller, J.N. Alpha-synuclein alters proteasome function, protein synthesis, and stationary phase viability. *J Biol Chem* **280**, 30009-17 (2005).
36. Dixon, C., Mathias, N., Zweig, R.M., Davis, D.A. & Gross, D.S. Alpha-synuclein targets the plasma membrane via the secretory pathway and induces toxicity in yeast. *Genetics* **170**, 47-59 (2005).
37. Flower, T.R., Chesnokova, L.S., Froelich, C.A., Dixon, C. & Witt, S.N. Heat shock prevents alpha-synuclein-induced apoptosis in a yeast model of Parkinson's disease. *J Mol Biol* **351**, 1081-100 (2005).
38. Outeiro, T.F. & Lindquist, S. Yeast cells provide insight into alpha-synuclein biology and pathobiology. *Science* **302**, 1772-5 (2003).
39. Sharma, N. et al. alpha-Synuclein budding yeast model: toxicity enhanced by impaired proteasome and oxidative stress. *J Mol Neurosci* **28**, 161-78 (2006).
40. Winderickx, J. et al. Protein folding diseases and neurodegeneration: lessons learned from yeast. *Biochim Biophys Acta* **1783**, 1381-95 (2008).
41. Outeiro, T. Yeast Cells Provide Insight into Alpha-Synuclein Biology and Pathobiology. *Science* **302**, 1772-1775 (2003).
42. Su, L.J. et al. Su, L.J., Auluck, P.K., Outeiro T.F., Yeger-Lotem, E., Kritzer, J.A. , D.F. Tardiff et al. *Disease Models and Mechanisms* **In press** (2009).
43. Chopra, V. et al. A small-molecule therapeutic lead for Huntington's disease: preclinical pharmacology and efficacy of C2-8 in the R6/2 transgenic mouse. *Proc Natl Acad Sci U S A* **104**, 16685-9 (2007).
44. Schaffar, G. et al. Cellular toxicity of polyglutamine expansion proteins: mechanism of transcription factor deactivation. *Mol Cell* **15**, 95-105 (2004).
45. Duennwald, M.L., Jagadish, S., Giorgini, F., Muchowski, P.J. & Lindquist, S. A network of protein interactions determines polyglutamine toxicity. *Proc Natl Acad Sci U S A* **103**, 11051-6 (2006).
46. Duennwald, M.L., Jagadish, S., Muchowski, P.J. & Lindquist, S. Flanking sequences profoundly alter polyglutamine toxicity in yeast. *Proc Natl Acad Sci USA* **103**, 11045-50 (2006).
47. Gitler, A.D. et al. Alpha-synuclein is part of a diverse and highly conserved interaction network that includes PARK9 and manganese toxicity. *Nat Genet* **41**, 308-15 (2009).
48. Jankovic, J. Searching for a relationship between manganese and welding and Parkinson's disease. *Neurology* **64**, 2021-8 (2005).
49. Ramirez, A. et al. Hereditary parkinsonism with dementia is caused by mutations in ATP13A2, encoding a lysosomal type 5 P-type ATPase. *Nat Genet* **38**, 1184-91 (2006).
50. Schmidt, K., Wolfe, D.M., Stiller, B. & Pearce, D.A. Cd2+, Mn2+, Ni2+ and Se2+ toxicity to Saccharomyces cerevisiae lacking YPK9p the orthologue of human ATP13A2. *Biochemical and Biophysical Research Communications* **383**, 198-202 (2009).
51. Yeger-Lotem, E. et al. Bridging high-throughput genetic and transcriptional data reveals cellular responses to alpha-synuclein toxicity. *Nat Genet* **41**, 316-23 (2009).
52. Wahner, A.D., Bronstein, J.M., Bordelon, Y.M. & Ritz, B. Statin use and the risk of Parkinson disease. *Neurology* **70**, 1418-22 (2008).
53. Willingham, S., Outeiro, T.F., DeVit, M.J., Lindquist, S.L. & Muchowski, P.J. Yeast genes that enhance the toxicity of a mutant huntingtin fragment or alpha-synuclein. *Science* **302**, 1769-72 (2003).
54. Zhang, X. et al. A potent small molecule inhibits polyglutamine aggregation in Huntington's disease neurons and suppresses neurodegeneration in vivo. *Proc Natl Acad Sci USA* **102**, 892-7 (2005).
55. Marsh, J.L. & Thompson, L.M. Drosophila in the study of neurodegenerative disease. *Neuron* **52**, 169-78 (2006).
56. Tam, S., Geller, R., Spiess, C. & Frydman, J. The chaperonin TRiC controls polyglutamine aggregation and toxicity through subunit-specific interactions. *Nat Cell Biol* **8**, 1155-62 (2006).
57. Vacher, C., Garcia-Oroz, L. & Rubinsztein, D.C. Overexpression of yeast hsp104 reduces polyglutamine aggregation and prolongs survival of a transgenic mouse model of Huntington's disease. *Hum Mol Genet* **14**, 3425-33 (2005).

58. Carnemolla, A. et al. Rrs1 is involved in endoplasmic reticulum stress response in huntington disease. *J Biol Chem* **284**, 18167-73 (2009).
59. Sas, K., Robotka, H., Toldi, J. & Vécsei, L. Mitochondria, metabolic disturbances, oxidative stress and the kynurenine system, with focus on neurodegenerative disorders. *J Neurol Sci* **257**, 221-39 (2007).
60. Arrasate, M., Mitra, S., Schweitzer, E.S., Segal, M.R. & Finkbeiner, S. Inclusion body formation reduces levels of mutant huntingtin and the risk of neuronal death. *Nature* **431**, 805-10 (2004).
61. Treusch, S., Cyr, D.M. & Lindquist, S. Amyloid deposits: protection against toxic protein species? *Cell Cycle* **8**, 1668-74 (2009).
62. Ehrnhoefer, D.E. et al. Green tea (-)-epigallocatechin-gallate modulates early events in huntingtin misfolding and reduces toxicity in Huntington's disease models. *Hum Mol Genet* **15**, 2743-51 (2006).
63. Gitler, A.D. et al. The Parkinson's disease protein alpha-synuclein disrupts cellular Rab homeostasis. *Proc Natl Acad Sci USA* **105**, 145-50 (2008).
64. Soper, J.H. et al. Alpha-synuclein-induced aggregation of cytoplasmic vesicles in Saccharomyces cerevisiae. *Mol Biol Cell* **19**, 1093-103 (2008).
65. Flower, T.R. et al. YGR198w (YPP1) targets A30P alpha-synuclein to the vacuole for degradation. *J Cell Biol* **177**, 1091-104 (2007).
66. Griffioen, G. et al. A yeast-based model of alpha-synucleinopathy identifies compounds with therapeutic potential. *Biochim Biophys Acta* **1762**, 312-8 (2006).
67. Kritzer, J.A. et al. Rapid selection of cyclic peptides that reduce alpha-synuclein toxicity in yeast and animal models. *Nat Chem Biol* **5**, 655-63 (2009).
68. Larsen, K.E. et al. Alpha-synuclein overexpression in PC12 and chromaffin cells impairs catecholamine release by interfering with a late step in exocytosis. *J Neurosci* **26**, 11915-22 (2006).
69. Jones, G.M. et al. A systematic library for comprehensive overexpression screens in Saccharomyces cerevisiae. *Nat Methods* **5**, 239-41 (2008).
70. DeRisi, J.L., Iyer, V.R. & Brown, P.O. Exploring the metabolic and genetic control of gene expression on a genomic scale. *Science* **278**, 680-6 (1997).
71. Alberti, S., Gitler, A.D. & Lindquist, S. A suite of Gateway ®cloning vectors for high-throughput genetic analysis in Saccharomyces cerevisiae. *Yeast* **24**, 913-919 (2007).
72. http://www.addgene.org/yeast_gateway.
73. Ejsing, C.S. et al. Global analysis of the yeast lipidome by quantitative shotgun mass spectrometry. *Proc Natl Acad Sci USA* **106**, 2136-41 (2009).
74. Gaspar, M.L. et al. The emergence of yeast lipidomics. *Biochim Biophys Acta* **1771**, 241-54 (2007).
75. Hoon, S., St Onge, R.P., Giaever, G. & Nislow, C. Yeast chemical genomics and drug discovery: an update. *Trends Pharmacol Sci* **29**, 499-504 (2008).
76. Parsons, A.B. et al. Exploring the mode-of-action of bioactive compounds by chemical-genetic profiling in yeast. *Cell* **126**, 611-25 (2006).
77. Hillenmeyer, M.E. et al. The chemical genomic portrait of yeast: uncovering a phenotype for all genes. *Science* **320**, 362-5 (2008).
78. Outeiro, T.F. & Giorgini, F. Yeast as a drug discovery platform in Huntington's and Parkinson's diseases. *Biotechnol J* **1**, 258-69 (2006).
79. Pearce, D.A., Ferea, T., Nosel, S.A., Das, B. & Sherman, F. Action of BTN1, the yeast orthologue of the gene mutated in Batten disease. *Nat Genet* **22**, 55-8 (1999).
80. Zhang, S. et al. Ncr1p, the yeast ortholog of mammalian Niemann Pick C1 protein, is dispensable for endocytic transport. *Traffic* **5**, 1017-30 (2004).
81. Moore, J.K., Sept, D. & Cooper, J.A. Neurodegeneration mutations in dynactin impair dynein-dependent nuclear migration. *Proc Natl Acad Sci U S A* **106**, 5147-52 (2009).
82. Pearce, D.A. Hereditary spastic paraplegia: mitochondrial metalloproteases of yeast. *Hum Genet* **104**, 443-8 (1999).
83. Shani, N. & Valle, D. A Saccharomyces cerevisiae homolog of the human adrenoleukodystrophy transporter is a heterodimer of two half ATP-binding cassette transporters. *Proc Natl Acad Sci U S A* **93**, 11901-6 (1996).
84. Middendorp, O., Lüthi, U., Hausch, F. & Barberis, A. Searching for the most effective screening system to identify cell-active inhibitors of beta-secretase. *Biol Chem* **385**, 481-5 (2004).
85. Bagriantsev, S. & Liebman, S. Modulation of Abeta42 low-n oligomerization using a novel yeast reporter system. *BMC Biol* **4**, 32 (2006).

86. von der Haar, T., Jossé, L., Wright, P., Zenthon, J. & Tuite, M.F. Development of a novel yeast cell-based system for studying the aggregation of Alzheimer's disease-associated Abeta peptides in vivo. *Neuro-degenerative diseases* **4**, 136-47 (2007).
87. Caine, J. et al. Alzheimer's Abeta fused to green fluorescent protein induces growth stress and a heat shock response. *FEMS Yeast Res* **7**, 1230-6 (2007).
88. Bharadwaj, P., Waddington, L., Varghese, J. & Macreadie, I.G. A new method to measure cellular toxicity of non-fibrillar and fibrillar Alzheimer's Abeta using yeast. *J Alzheimers Dis* **13**, 147-50 (2008).
89. Johnson, B.S., McCaffery, J.M., Lindquist, S. & Gitler, A.D. A yeast TDP-43 proteinopathy model: Exploring the molecular determinants of TDP-43 aggregation and cellular toxicity. *Proc Natl Acad Sci USA* **105**, 6439-44 (2008).
90. Apodaca, J., Kim, I. & Rao, H. Cellular tolerance of prion protein PrP in yeast involves proteolysis and the unfolded protein response. *Biochemical and Biophysical Research Communications* **347**, 319-26 (2006).
91. Ma, J. & Lindquist, S. De novo generation of a PrPSc-like conformation in living cells. *Nat Cell Biol* **1**, 358-61 (1999).
92. Tank, E.M., Harris, D.A., Desai, A.A. & True, H.L. Prion protein repeat expansion results in increased aggregation and reveals phenotypic variability. *Mol Cell Biol* **27**, 5445-55 (2007).
93. Yang, W., Yang, H. & Tien, P. In vitro self-propagation of recombinant PrPSc-like conformation generated in the yeast cytoplasm. *FEBS Lett* **580**, 4231-5 (2006).