PERSPECTIVES

OPINION

Modelling neurodegeneration in *Saccharomyces cerevisiae*: why cook with baker's yeast?

Vikram Khurana and Susan Lindquist

Abstract | In ageing populations, neurodegenerative diseases 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 to develop novel therapeutics. Here, we describe why the budding yeast *Saccharomyces cerevisiae* has a unique role in the neurodegeneration armamentarium. As the best-understood and most readily analysed eukaryotic organism, *S. cerevisiae* is delivering mechanistic insights into cell-autonomous mechanisms of neurodegeneration at an interactome-wide scale.

Neurodegenerative diseases are among the most pressing public health challenges facing the ageing 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. Although the neuropathologic observations have been instrumental in identifying pathologic proteinacious aggregates and patterns of differential neuronal vulnerability, they cannot distinguish causal from epiphenomenal factors in disease pathogenesis. Over the past 15 years we have gained tremendous insights 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 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 from the study of these model systems will be relevant to the study and treatment of human disease. For example, the identification and characterization of an upstream causative role for amyloid-β production in Alzheimer's disease (AD) has been a concerted effort in which neuropathology, biochemistry, human genetics and work within model systems, including fruitflies, worms and mice, have each had key roles. It is becoming clear that multiple complementary approaches will be needed to make substantial strides towards developing therapies.

In a research community becoming accustomed to a plethora of model systems, it nevertheless remains surprising to many that complex disease processes, such as those that contribute 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 on an unprecedented scale.

The yeast model system

Two general characteristics confer suitability upon any model system for the study of human disease: the model 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]. Although 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 pair-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 6,600 open reading frames (ORFs; protein-encoding genomic sequences) have been annotated, with more than 80% functionally characterized (see the *Saccharomyces* Genome Database). At least 60% of yeast genes have statistically robust human homologues or at least one conserved domain with human genes[1,4]. In addition, more than 25% of positionally cloned human disease genes have a close yeast homologue[5,6]. Genomic homology explains the conservation of fundamental cell biological processes between yeast and mammalian cells. Yeast cells, like mammalian cells, are eukaryotic and are distinguished from bacteria and archaea 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 homologues (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 in neurons, leading to neurodegeneration. Yeast prions were identified as non-Mendelian elements of inheritance that, like mammalian prions, alter cellular phenotype through a protein-only mode of transmission[7]. 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 shows similar biochemical properties to amyloid in neurodegenerative diseases, including recognition by Congo Red and thioflavin T, β-strands running perpendicular to the fibre 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[8].

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 the fission and fusion of mitochondria. Genetic defects in this machinery have now been causally linked to neurodegenerative disease[9].

The secretory pathway, through which proteins are translocated from the endoplasmic reticulum (ER) to the Golgi complex 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 by vesicular fusion. Our understanding of the molecular mechanisms of this pathway owes a great deal to yeast, in which screening for mutants that cause accumulation of secretory proteins resulted in the discovery of 23 SEC genes that encode the central components of the secretory and vesicular trafficking machinery[10]. Yeast has homologues of synaptobrevin, syntaxin and synaptosomal-associated protein 25 (SNAP25) among other key mammalian components of this pathway[11]. Importantly, ER stress caused by the accumulation of misfolded proteins in vesicular trafficking has been heavily implicated in neurodegeneration and cerebral white matter disease[12–14].

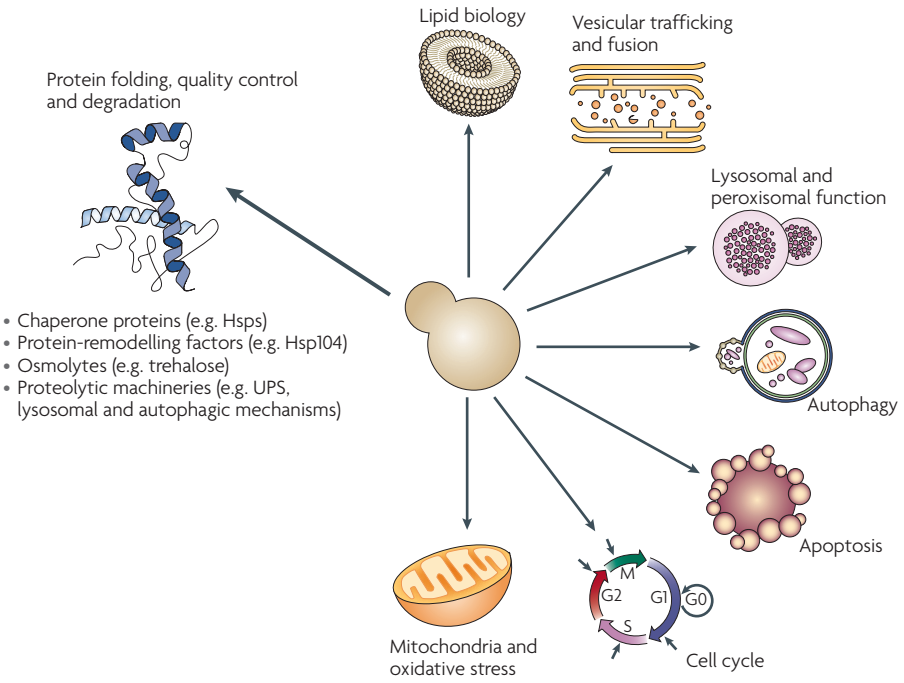


Figure 1 | **Conserved cellular biology in yeast.** Numerous cellular pathways of high relevance to neurodegeneration are conserved in yeast. Indeed, many owe their characterization to molecular dissection in the yeast model system (see main text for details). Although there are undoubtedly conserved apoptotic mechanisms in yeast, the presence of a classical programmed cell death pathway remains controversial. Given the fundamental role of protein misfolding, quality control and degradation machineries in neurodegenerative disease, their conservation is particularly pertinent. In yeast, chaperone proteins (such as the heat-shock proteins Hsp40, Hsp60, Hsp70 and Hsp90) and their cofactors bind to specific protein-folding intermediates, maintain their soluble state and promote their maturation. Protein-remodelling factors (such as Hsp104 in yeast, the orthologue of mammalian p97) and osmolytes (such as trehalose, betaine and triethylamine *N*-oxide) serve as 'chemical chaperones' by changing the hydrodynamic interactions between proteins and promoting maintenance of the folded state. These factors and osmolytes interact with improperly folded proteins and rearrange them using ATP. Proteolytic machineries, such as the ubiquitin–proteasome system (UPS), chaperone-mediated autophagy and the autophagy–lysosomal system, selectively degrade unstable and misfolded proteins. Membrane-bound compartments (including the nuclear envelope, mitochondria, Golgi complex, endoplasmic reticulum, lysosomes and endosomes) segregate distinct, functionally related proteins and the vesicular-trafficking machinery. Components of the vesicular-trafficking machinery include SNARE (soluble NSF (*N*-ethylmaleimide-sensitive factor) attachment protein (SNAP) receptor), clathrin, COPI (coat protein I), COPII and the ESCRT (endosomal sorting complex required for transport) complexes.

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. Apoptotic and non-apoptotic cell death mechanisms have both been implicated in neurodegeneration. In addition, aberrant reactivation of highly conserved cell cycle mechanisms in post-mitotic neurons may lead to apoptosis in some contexts[15]. 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 phosphatidylserine at the plasma membrane and labelling by TUNEL (TdT-mediated dUTP nick-end labelling) staining[16]. There is accumulating evidence that fundamental molecular mechanisms of programmed cell death (PCD) may be conserved in yeast. Although the existence of a PCD 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[17]. Experiments on yeast 'apoptosis' have shown how molecular analysis of a process in yeast can be productive even when yeast homologues for particular genes do not exist. Despite the presence of apoptotic mediators in yeast cells, they lack the mammalian B cell leukaemia/lymphoma (Bcl)/Bcl-2-associated X (Bax) family of apoptotic regulators. Nevertheless, heterologous expression of mammalian Bcl/Bax family members in yeast reveals conserved modulation of cell survival. When BAX 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[18]. The conserved function exists presumably because yeast possesses Bcl-2-like proteins — with divergent sequences from their mammalian counterparts — that are functional homologues, such as

Box 1 | **'Yeastomics'**

Here, we list modern tools available in *Saccharomyces cerevisiae* that enable systematic analysis of a cellular process or a phenotype.

Functional genomics: genetic screening libraries

Systematic large-scale profiling of genetic interactions in a cell.

- ***Deletion mutant collection.*** A collection of ~6,000 heterozygous diploid strains, each deleted for a specific gene. In practice, a collection of 4,000 haploid strains, each deleted for a non-essential gene, has 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 (for example, conditional mutants or gene manipulations to lower transcript levels).
- ***Comprehensive gene overexpression libraries.*** Libraries containing the complete set of yeast open reading frames (ORFs) 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 5,000 sequence-verified ORFs.
- ***Genomic plasmid libraries.*** Libraries encompassing both the protein-coding and non-coding genome, offering the advantage of containing genes under the control of their endogenous promoter[52].

Transcriptomics

Systematic large-scale profiling of the expression level of mRNAs in a given cell population. *S. cerevisiae* was the first organism for which cDNA microarrays were used for genome-wide transcriptional profiling[53], introducing a powerful method of generating insights into the biological effects of genetic, chemical and environmental manipulation. Profiles now exist for thousands of manipulations.

Proteomics

Systematic large-scale profiling of the proteins of a cell.

- ***Yeast two-hybrid interaction screens.*** A protein of interest fused to the DNA-binding domain of the transcriptional activator galactose 4 (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.*** Fusions between a marker (such as green fluorescent protein (GFP), DsRed or glutathione *S*-transferase (GST)) and a protein allowing protein localization and the recovery and analysis of individual proteins and protein complexes. Such fusions were recently incorporated into a collection of vectors into which a yeast ORF can be readily introduced[54] (see *S. cerevisiae* Advanced Gateway Destination Vectors on the Addgene website).
- ***Protein microarray.*** Functional arrays used to analyse the biochemical activities of deposited proteins. Nickel or nitrocellulose-coated slides containing tagged proteins can be used to analyse protein–protein, protein–lipid and protein–nucleic acid interactions[1].

Metabolomics

Systematic large-scale profiling of the small-molecule metabolites of a cell.

- ***Lipidomics.*** The quantification of almost the entire set of yeast lipids and lipid-related metabolites (the 'lipidome'), providing unprecedented power to study a class of molecules that is increasingly recognized as crucial in complex cellular processes, including signalling and vesicle trafficking[55,56].

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.*** Automated robotic screening (in 96-, 384- or 1536-well format) of a phenotype or process against a small-molecule library. This method is rendered cost effective by its large scale and short generation time.
- ***Chemical genetic profiling.*** Screening of a compound 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[57,58].
- ***Chemical genetic mapping.*** An extensive chemical genetic analysis of *S. cerevisiae* (created by testing more than 1,000 chemical and environmental perturbations on every strain of the non-essential deletion library). It provides an extraordinary resource for predicting drug targets and drug synergy[59].
- ***Cyclic peptide libraries.*** Libraries of small proteins with a ring architecture. Recently, libraries of millions of cyclic peptides have been generated *in vivo*, providing a largely unexplored chemical space for screening, and a system in which structure–activity relationships can be rapidly generated for the peptides using point mutagenesis[41].

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 measurements of: carbon, nitrogen, phosphorus and sulphur catabolism; osmotic, pH and chemical sensitivity; small-molecule and macromolecule biosynthesis; and cell surface structure and transport[60].

metacaspase 1 (Mca1, also known as Yca1), or that share a common function despite a 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 part in identifying the effector machinery of autophagy, which consists of the highly conserved ATG proteins downstream of the target of rapamycin (TOR) kinase[19]. 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[20].

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 therefore suited for high-throughput genetic and small-molecule screens. Most important is its genetic tractability: its DNA is easily transformed, and homologous recombination is efficient[21,22]. 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 yeast 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 analysed by genome-wide genetic interaction screens, transcriptional profiling, proteomic analysis, lipidomic analysis, small molecule and chemical–genetic analysis, and phenotype microarrays (BOX 1). The genetic tractability and genomic stability of yeast gives it a substantial advantage over transformed mammalian cell culture lines, and these features are particularly desirable in a model system for neurodegenerative diseases.

Yeast phenotypes can be screened against genome-wide deletion, reduced expression or overexpression libraries. Such libraries include collections of plasmids for manipulating the expression of every ORF of the yeast genome under the expression of different promoters (BOX 1). Automated screening of a phenotype against the entire overexpression library can be accomplished in weeks. Hundreds of genome-wide screens for synthetic-lethal genetic interactions (combinations of two mutations which themselves have little or no phenotypic effect, but which together are severely toxic or inviable) have been used extensively to identify proteins that 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. In addition, several large-scale proteomics projects using two-hybrid analysis and high-throughput mass spectroscopy have yielded ~45,000 protein–protein interactions, and thousands of expression profiles have been generated with diverse genetic, chemical and environmental perturbations. The wealth of interaction data have been compiled in publicly available databases (TABLE 1).

Table 1 | **A selection of online yeast databases**

Database	URL
Saccharomyces Genome Database (SGD)	http://www.yeastgenome.org
The Comprehensive Yeast Genome Database (from the Munich Information Center for Protein Sequences)	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)	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.yeastgenome.org
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

Limitations. As a unicellular organism with a cell wall, the most obvious limitation of yeast as a model system for neurodegenerative disease is in the analyses of disease aspects that rely on multicellularity and cell–cell interactions. Such interactions include immune and inflammatory responses, synaptic transmission and glial–neuronal interactions, among others.

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 simpler complex called external NADH dehydrogenase 2 (Ndh2, also known as Nde2) 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, the response is far more complex in mammalian cells[23]. Many neuronal specializations that are likely to be of great importance to neurodegeneration — for example, axonal transport, neurotransmitter release and myelination — cannot be recapitulated in yeast. Nevertheless, fundamental aspects of these biological functions may be conserved in yeast. For example, although yeast

cells do not release neurotransmitters, they traffic proteins in vesicles and have conserved endo- and exocytic mechanisms and, although yeast cells do not produce myelin, they have 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. However, the important biological differences between yeast and complex diseases require that insights gained from the yeast system be validated in neuronal model systems.

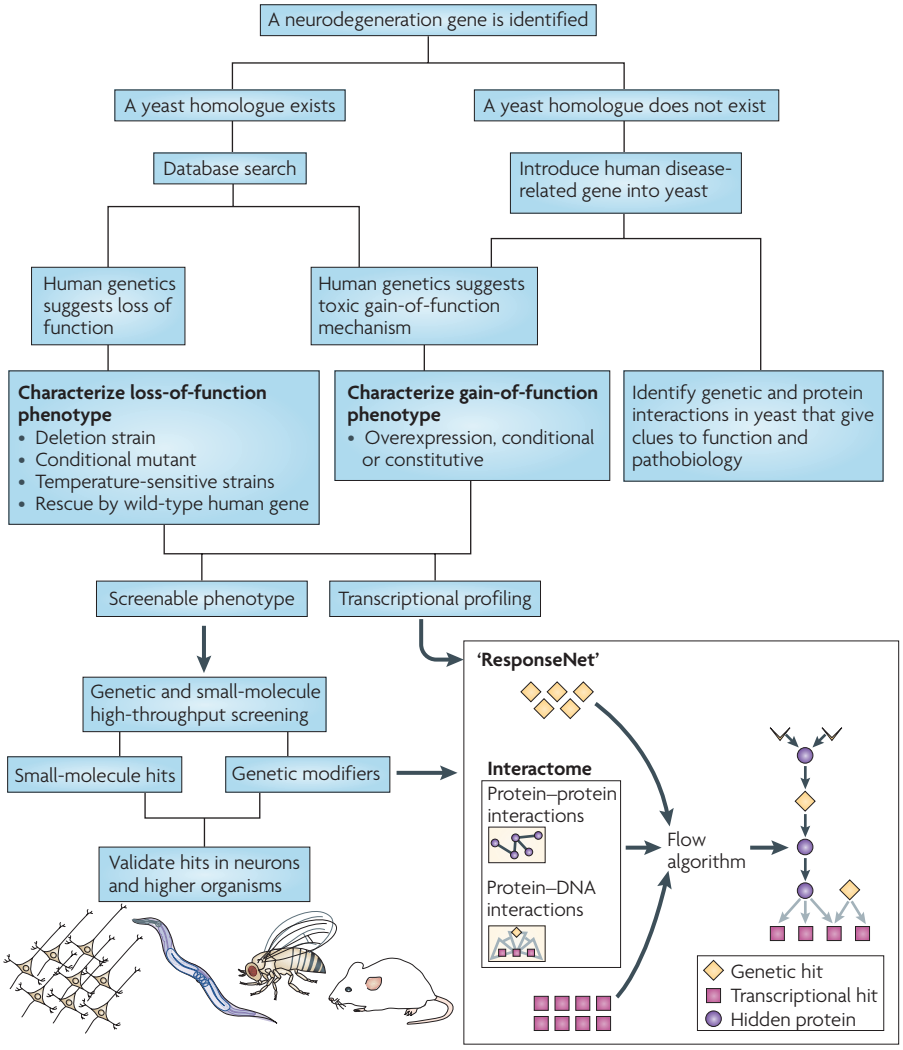


Figure 2 | **Creating, characterizing and screening a yeast model of neurodegenerative disease.** When a neurodegenerative disease-causing mutation is identified, the first task is to find out whether a homologue exists in yeast by consulting existing yeast databases (TABLE 1). If a yeast homologue exists, there may be substantial characterization of the gene in question. Loss-of-function genes can be characterized by gene deletion and conditional mutants. Complementation is established when a human gene can 'rescue' the loss-of-function phenotype in yeast. If a homologue does not exist, the human gene product can be introduced into yeast. This can be purely to analyse its function by harnessing the yeast toolbox (genetic and protein interactions) or, in cases in which human genetics suggests a toxic gain-of-function mechanism, overexpression of the gene in yeast may recapitulate cellular pathology associated with the disease. After a yeast model has been created by either gene disruption or overexpression, analysis proceeds by profiling — most commonly transcriptional profiling — or 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 to uncover unsuspected components of pathways (see main text). Genetic modifiers and small-molecule hits from yeast screens are validated in cultured or whole-organism neuronal systems. Cultured neuronal systems include rodent primary cultures and, in the future, will include novel induced pluripotential stem cell-derived neurons from patients.

Yeast neurodegeneration models

Modelling human disease in yeast follows one of two general approaches, depending on whether a yeast homologue exists (FIG. 2). When a human disease-related gene has a yeast homologue, the gene can be disrupted or overexpressed to determine the loss- or gain-of-function phenotypes, respectively[21,22]. Clear yeast homologues exist for many genes associated with neurodegeneration (a non-exhaustive list is provided in TABLE 2). 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, which is encoded by *FRDA* (also known as *FXN*). Studies of the yeast *FRDA* homologue *YFH1* have been instrumental in determining the function of frataxin. Yeast with *YFH* 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–sulphur cluster enzymes including aconitase. Importantly, human *FRDA* rescues these phenotypes. These findings in yeast were confirmed in mouse models and in fibroblasts from patients. Although the exact role of each of these cellular defects in pathology is debated, the antioxidant idebenone has shown promise for both neurologic and cardiac manifestations in early clinical trials[24,25].

For human disease-related genes that do not have a yeast homologue 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 disease (HD) and other polyglutamine (poly(Q)) diseases, synucleinopathies (such as PD), AD, amyotrophic lateral sclerosis, prion diseases and tauopathies (TABLE 3). Creating a cellular or animal model by ectopic expression of the implicated proteins is justifiable if overexpression recapitulates key features of the disease, if knocking out or reducing gene dosage does not recapitulate these features and, perhaps most convincingly, if the

Table 2 | **Neurodegenerative disease genes with *Saccharomyces cerevisiae* homologues**

Human	Yeast	Disease	Human protein product	Inheritance/comments
Ataxia				
FRDA (also known as *FXN*)*	*YFH1*	FA	Frataxin, a nuclear-encoded mitochondrial protein involved in iron–sulphur cluster enzyme biogenesis	Autosomal recessive, generally caused by expansion of an unstable GAA repeat in the first intron; FA is the most common inherited ataxia[24]
ATXN2	*PBP1*	SCA2	Ataxin 2, which has a putative role in RNA metabolism	Autosomal dominant, caused by an expanded polyglutamine tract
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
ATXN7	*SGF73*	SCA7	Ataxin 7, a subunit of histone acetyltransferase complexes	Autosomal dominant, caused by an expanded polyglutamine tract
ATXN10	*ATXN10*	SCA10	Ataxin 10	Autosomal dominant, caused by a pentanucleotide (ATTCT) repeat expansion
TBP	*SPT15*	SCA17	TATA-box-binding protein	Autosomal dominant SCA caused by an expanded polyglutamine tract
TDP1	*TDP1*	SCAN1	TDP1, a DNA-repair protein	Autosomal recessive
ATM	*TEL1*	AT	ATM, a key regulator of the DNA-damage response	Autosomal recessive
Dementia				
SORL1 (also known as *LR11*)	*PEP1*, *VTH1*, *VTH2*, *YNR065C*	AD	Sortilin 1, a neuronal sorting receptor of the LDL receptor family	Genetically associated with late-onset AD
CHMP2B	*DID4*	FTD	CHMP2B, an ESCRTIII subunit important in endosomal–lysosomal trafficking	Autosomal dominant
Lysosomal storage disease				
CTSD	*PEP4*	NCL	Cathepsin D, a lysosomal protease	Autosomal recessive congenital NCL
*CLN3**	*YHC3* (also known as *BTN1*)	NCL (BD)	CLN3, a lysosomal transmembrane protein	Autosomal recessive juvenile NCL (also known as BD)[78]
SMPD1	*PPN1*	NP type A	Sphingomyelin phosphodiesterase, a lysosomal enzyme	Autosomal recessive
*NPC1**	*NCR1*	NP type C	NPC1, a late endosomal protein involved in cholesterol trafficking	Autosomal recessive, associated with intraneuronal accumulation of unesterified cholesterol[79]
Upper/lower motor neuron disease				
SOD1	*SOD1*	ALS	Superoxide dismutase, a major antioxidant enzyme	Autosomal dominant
VAPB	*SCS2*, *SCS22*	SMA, ALS, FTD-ALS	VAPB, a membrane protein involved in vesicle trafficking	Autosomal dominant
*DCN1**	*NIP100*	ALS	p150 subunit of dynactin, a protein involved in axonal transport	Autosomal dominant (probably a dominant negative mutation)[80]
Upper motor neuron disease				
REEP1	*YOP1*	HSP	REEP1, a mitochondrial protein	Autosomal dominant
*SPG7**	*AFG3*, *RCA1*	HSP	Paraplegin, a mitochondrial AAA protease	Autosomal recessive, leads to axonal degeneration of corticospinal tracts and dorsal columns[81]
Movement disorder				
DJ1 (also known as *PARK7*)	*HSP31*	PD	DJ1, a redox-activated chaperone	Autosomal recessive parkinsonism
PANK2	*CAB1*	PKAN	Pantothenate kinase 2, involved in CoA biosynthesis	Autosomal recessive parkinsonism and brain iron accumulation
UCHL1	*YUH1*	PD?	UCHL1, a protein of unclear function	Controversial susceptibility gene for familial PD; modulates toxicity in an AD mouse model
VPS13A	*VPS13*	NA	Chorein, which has putative involvement in membrane protein trafficking	Autosomal recessive movement disorder associated with acanthocytes in the blood and degeneration of the basal ganglia

Table 2 (cont.) | **Neurodegenerative disease genes with *Saccharomyces cerevisiae* homologues**

Human	Yeast	Disease	Human protein product	Inheritance/comments
Peripheral neuropathy				
GARS	*GRS1*, *GRS2*	CMT2D, distal SMA type V	Glycyl-tRNA synthetase	Autosomal dominant degeneration of peripheral sensory and motor neurons (CMT2D) or just motor neurons (distal SMA type V)
GDAP1	*GTT2*	CMT4A	GDAP1	Autosomal recessive degenerative disease of peripheral motor and sensory neurons
MTMR2, *SBF2*	*YMR1*	CMT4B1, CMT4B2	MTMR2 and MTMR13 are myotubularin-related proteins linked with vesicular trafficking	Autosomal recessive
FIG4	*FIG4*	CMT4J	FIG4, a phosphatase in the vacuolar membrane associated with endosome–lysosome trafficking	Autosomal recessive
YARS	*TYS1*	DICMT-C	Tyrosyl-tRNA synthetase	Autosomal dominant degeneration of peripheral sensory and motor neurons
SPTLC1	*LCB1*	HSN	Serine palmitoyltransferase 1, which is involved in sphingolipid synthesis	Autosomal dominant degeneration of peripheral sensory neurons
White matter disease				
*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[82]
PEX1, *PEX5*, *PEX10*, *PEX13*, *PEX14*, *PEX19*	*PEX1*, *PEX5*, *PEX10*, *PEX13*, *PEX14*, *PEX19*	Zellweger syndrome	Peroxisome biogenesis proteins	Autosomal recessive degenerative white matter disease
XPC, *ERCC2*, *DDB1*, *DDB2*, *RAD1*, *RAD30*	*RAD4*, *RAD3*, *RSE1* (also known as *SF3B3*), *CMR1*, *ERCC4*, *POLH*	XP type C, XP type D, 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 and deafness

This is a partial list that was created by BLAST Ensembl alignment. Many genes with less sequence homology than those listed here may still have highly conserved domain homology and function. *Genes that have been used to create yeast models (see references for more details). ABCD1, ATP-binding cassette, subfamily D, member 1; AD, Alzheimer's disease; ALD, adrenoleukodystrophy; ALS, amyotrophic lateral sclerosis; AT, ataxia telangiectasia; ATM, ataxia telangiectasia mutated; BD, Batten disease; CACNA1A, calcium channel, voltage-dependent, P/Q type, α1A subunit; CHMP2B, chromatin-modifying protein 2B; CLN3, ceroid-lipofuscinosis, neuronal 3; CMT, Charcot–Marie–Tooth disease; DDB, damage-specific DNA binding; DICMT, dominant intermediate CMT; ERCC2, excision repair cross-complementing rodent repair deficiency, complementation group 2; ESCRTIII, endosomal sorting complex required for transport III; FA, Friedreich's ataxia; FTD, frontotemporal dementia; GDAP1, ganglioside-induced differentiation-associated protein 1; HSN, hereditary sensory neuropathy; HSP, hereditary spastic paraplegia; *HSP31*, heat-shock protein 31; NA, neuroacanthocytosis; NCL, neuronal ceroid lipofuscinosis; NP, Niemann–Pick disease; PD, Parkinson's disease; PEP, peptidase; PKAN, pantothenate kinase-associated neurodegeneration; REEP1, receptor expression-enhancing protein 1; SBF2, SET-binding factor-2 (also known as MTMR13); SCA, spinocerebellar ataxia; SCAN, spinocerebellar ataxia with axonal neuropathy; SMA, spinal muscular atrophy; SPG7, spastic paraplegia 7; SPTLC1, serine palmitoyltransferase, long chain base subunit 1; TDP1, tyrosyl-DNA phosphodiesterase 1; UCHL1, ubiquitin carboxyl-terminal esterase L1; VAPB, vesicle-associated membrane protein B; VPS, vacuolar protein sorting; XP, xeroderma pigmentosum.

human disease can be caused by inheriting extra copies of the encoding gene. In the following sections (and in BOX 2 and BOX 3), we describe two yeast models that recapitulate key cellular pathologies of human disease and that have led to important mechanistic insights which have subsequently been validated in neuronal model systems.

Yeast model of polyglutamine expansion disorders. Poly(Q) expansion disorders are autosomal-dominant disorders caused by a CAG repeat expansion in the ORF that encodes a protein with an expanded poly(Q) tract. A poly(Q) tract must exceed a critical length, usually ~35 glutamines, to initiate disease, and longer tracts generally cause 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, HD[25]. HD is characterized by hyperkinetic movements, psychosis and cognitive dysfunction and is caused by poly(Q) expansion in the amino-terminal end of the huntingtin (HTT) protein, leading to loss of striatal medium spiny neurons and cortical neurons. HTT exon 1 (HTTEX1) is found in the neuronal aggregates of HD and its overexpression is sufficient to produce neurodegeneration in mouse models[26]. The yeast model was made by overexpressing fragments of HTTEX1 followed by poly(Q) tracts of different lengths (BOX 2). Phenotypes include poly(Q) length-dependent HTTEX1 aggregation and the formation of higher-order complexes[27–30], impaired growth accompanied by mitochondrial dysfunction and oxidative stress, apoptosis-like DNA fragmentation[31,32], transcriptional dysregulation[27], and a dramatic deficit of ER-associated degradation (ERAD), which is linked to an unfolded protein response[33].

Yeast model of synucleinopathy. 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 type 1. PD is characterized clinically by parkinsonism (bradykinesia, rigidity and resting tremor) and responsiveness to 3,4-dihydroxyphenylalanine DOPA (L-DOPA) therapy, and pathologically by neuronal α-syn inclusions

Table 3 | **Neurodegenerative diseases caused by toxic gain-of-function mechanisms**

Disease	Aggregating protein	Yeast model	Phenotype	Refs
AD	Aβ, cleaved from APP through the action of two enzymes, β- and γ-secretase	APP co-expression with reconstituted β- and γ-secretase	Cleavage products of APP produced, allowing monitoring of protease activity and identification of small-molecule inhibitors of β-secretase	83
		Aβ expressed as a fusion protein with the yeast prion Sup35p	Sup35p reporter gene function serves as a surrogate for Aβ aggregation	84,85
		Aβ expressed as a fusion protein with GFP	Inhibition of growth and heat-shock response	86
		Exogenous Aβ applied to yeast, fibrillar compared with non-fibrillar/oligomeric Aβ	Non-fibrillar/oligomeric Aβ causing enhanced loss of cell viability compared with fibrillar Aβ	87
Synucleinopathies, including PD	α-Synuclein	Expression of α-synuclein	Cytosolic aggregation of α-synuclein associated with vesicular membrane; oxidative stress, lipid-droplet accumulation, vesicle-trafficking defects and mitochondrial perturbations	12, 34–39
Polyglutamine expansion diseases, including HD	Many proteins with polyglutamine expansions, including huntingtin	Expression of huntingtin exon 1 with a polyglutamine tract of various lengths	Cytoplasmic aggregation, ERAD defects and proteasome impairment with expanded tracts (for example, 103Q)	27–30
FTD-U and ALS	TDP43	Expression of TDP43 fused to GFP	Increased expression leads to redistribution from nucleus to cytoplasm with concomitant aggregation and inhibition of growth	88
Prion disease	PrP	Expression of PrP with or without a signal sequence or expression of PrP fused to yeast prions	Cytosolic PrP forms insoluble PrP^{Sc}, and is capable of self-propagation; growth impairment occurs in ERAD- or UPR-deficient yeast	89–92
Tauopathy, including AD, FTD-tau, PSP and CBD	Tau	Expression of human tau (three repeat and four repeat isoforms of wild-type tau, and P301L mutant tau)	Increased tau phosphorylation and aggregation, the latter enhanced by oxidative stress and mitochondrial dysfunction; decreased longevity	39

The table lists neurodegenerative diseases that are putatively caused by toxic gain-of-protein-function mechanisms associated with intraneuronal and intraglial protein aggregates. The diseases have been modelled in yeast by overexpression of these proteins. Aβ, amyloid-β; AD, Alzheimer's disease; APP, amyloid precursor protein; CBD, corticobasal degeneration; ERAD, endoplasmic reticulum-associated degradation; FTD-tau, frontotemporal dementia associated with tau-immunoreactive inclusions; FTD-U, frontotemporal dementia associated with ubiquitin-immunoreactive tau-immunonegative inclusions; GFP, green fluorescent protein; HD, Huntington's disease; PD, Parkinson's disease; PrP, prion protein; PrP^{Sc}, infectious isoform of PrP; PSP, progressive supranuclear palsy; TDP43, TAR DNA-binding protein 43; UPR, unfolded protein response.

known as Lewy bodies (LBs) and prominent loss of midbrain dopaminergic and other neuronal populations. Approximately 5% of DOPA-responsive parkinsonism is associated with mutations at various *PARK* loci causing parkinsonism with Mendelian inheritance. Mutations at the *SNCA* (also known as *PARK1*) locus 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 Mn^{2+}, Fe^{2+} and Cu^{2+}, in the pathogenesis of synucleinopathies.

Yeast models of synucleinopathy are created by expressing different forms of human α-syn in yeast[34–39] (BOX 3). In one model, α-syn (wild type or mutant) linked to green fluorescent protein (GFP) is expressed from a regulatable promoter at different expression levels. GFP does not alter the biology or toxicity of the protein in yeast, therefore 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 and cytotoxicity (growth inhibition and cell death). Key aspects of mammalian cellular pathology are recapitulated, including defective vesicle trafficking and proteasomal degradation, ROS, mitochondrial pathology and lipid-droplet accumulation[36,37,39,40]. 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[36]. Microarray analysis at different times after α-syn induction implicated early mitochondrial dysfunction and ER stress, followed by vesicle trafficking and sterol biosynthesis[40]. An analysis using conditional *SEC* mutants revealed α-syn is trafficked to the plasma membrane through the classical secretory pathway[35].

Genetic and small-molecule analysis: important themes and validation in neuronal systems. As noted above, the 'humanized' yeast models for poly(Q) 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 cellular toxicity, and for high-throughput screens. In BOX 2 and BOX 3 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 overexpressed. For example, contributors to poly(Q) toxicity include 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 in both models. Small-molecule screens are identifying compounds that can reverse cellular defects in these models. For example, BOX 3 details compounds identified in a recent screen of ~115,000 compounds and ~5 million cyclic peptides that rescue cellular toxicity in a synucleinopathy model[40,41]. 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, which was identified in a yeast screen for small molecules that reduce poly(Q) aggregation. C2-8 has recently been shown to ameliorate neurodegeneration and motor deficits in a mouse model of HD[42]. Below, we offer select examples that show 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 poly(Q) expansion and protein misfolding, poly(Q) diseases

Box 2 | **A yeast model of Huntington's disease and other polyglutamine expansion disorders**

In the yeast strains shown in the figure, green fluorescent protein (GFP)-tagged huntingtin exon 1 (Httex1) is expressed under a regulatable (galactose (Gal)-inducible) promoter with different polyglutamine (poly(Q)) tract lengths. Immunofluorescence (left panels) shows that the intracytoplasmic aggregates form with higher tract lengths. A spot assay (fivefold serial dilution starting with equal numbers of cells; right panel) shows decreased growth with higher tract lengths (the glucose 'off' control is not shown).

Genetic modifiers

- ***Candidate testing.*** An initial study identified molecular chaperones and related proteins as important modulators of aggregate seeding and expansion[29]. Suppressors included chaperones and protein-remodelling factors — such as TCIP1 ring complex (TRiC), heat-shock protein 104 (Hsp104) and small heat-shock proteins — and the essential endoplasmic reticulum (ER)-associated degradation (ERAD) proteins nuclear protein localization 4 (Npl4) and ubiquitin fusion degradation 1 (Ufd1). The results for ERAD proteins suggested a causal relationship between ERAD dysfunction and Htt-induced toxicity in this model[33].
- ***Gene-deletion genome-wide suppressor screen (Httex1–103Q).*** This screen identified suppressors with roles in vesicle transport, vacuolar degradation and transcription. A large number of these contained glutamine- and asparagine-rich regions, and some are yeast prions. Biosynthesis of nicotinic acid 4 (Bna4, also known as kynurenine 3-monooxygenase), a mitochondrial enzyme in the kynurenine pathway of tryptophan degradation, was a potent suppressor and was essential for reactive oxygen species (ROS) generation in the yeast model. This study also showed that Httex1 aggregation occurs in several gene-deletion strains that suppress toxicity, disconnecting toxicity from the formation of large aggregates[31].
- ***Synthetic lethality enhancer screen (Httex1–53Q).*** This screen identified proteins involved in the response to stress (including some proteins required for the redox/oxidative stress response in yeast, such as glutathione synthase), protein folding and ubiquitin-dependent protein catabolism[61].

Small-molecule screening

- ***Aggregation of poly(Q)–GFP.*** Aggregation of poly(Q)–GFP has been used 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 crucial to toxicity).
- ***16,000-compound library screen.*** A 16,000-compound library screen yielded a potent inhibitor of aggregation, C2-8 (REF. 62).
- ***Epigallocatechin 3-gallate inhibits aggregation.*** A screen for ~5,000 natural substances revealed that the green tea polyphenol (–)-epigallocatechin 3-gallate (EGCG) potently inhibited the aggregation of poly(Q)-expanded Httex1 in a dose-dependent manner, and this was verified in yeast and higher model systems[39,63].

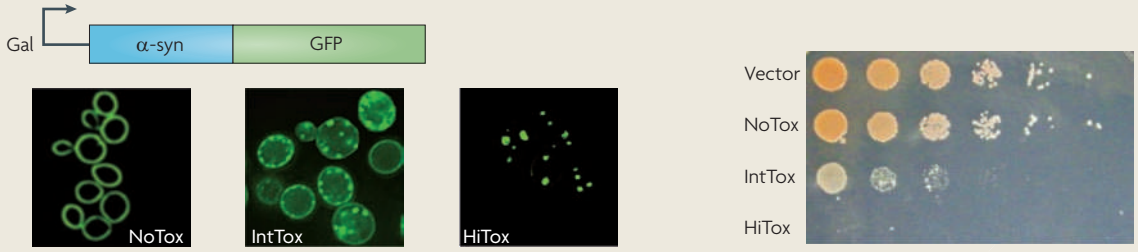
Validation in higher model systems

- ***Prolonged survival.*** The chaperones Hsp70, Hsp40 and TRiC and the protein-remodelling factor Hsp104 reduced the aggregation of Httex1 fragments *in vitro* and prolonged survival in flies, mice and mammalian cell models of Huntington's disease (HD)[30,64–66].
- ***Endoplasmic reticulum stress.*** ER stress was shown to be an early presymptomatic event that persists throughout life in an HD mouse model and in post-mortem brain tissue of patients with HD[67], validating the finding in yeast of poly(Q)-dependent ER stress and ERAD inhibition.
- ***Oxidative stress.*** Dysfunction of the kynurenine pathway was linked to oxidative stress in animal models of HD and in patients with HD[68], validating the finding that toxicity and ROS generation in the yeast HD model is dependent on Bna4 (REF. 31).
- ***Disconnection between genetic modifiers and aggregation.*** The clear disconnection in yeast screens between genetic modifiers of toxicity and the extent of Httex1 aggregation, implying that large aggregates are themselves non-pathogenic, has been validated in multiple model systems, including primary neuronal culture and higher organisms[69,70].
- ***Therapeutic compounds.*** Potential therapeutic compounds identified in yeast are being validated in higher model systems. C2-8 inhibited aggregation of mutant Htt in neurons in a hippocampal slice culture from a mouse HD model, ameliorated neurodegeneration in a *Drosophila melanogaster* HD model and rescued motor deficits and neurodegeneration in a mouse HD model[42]. Interestingly, C2-8 is a structural analogue of the compound Ro 61-8048, a high-affinity small-molecule inhibitor of Bna4, underscoring the genetic data linking this pathway to toxicity. EGCG rescued photoreceptor degeneration and motor function in HD transgenic flies overexpressing poly(Q)-expanded Httex1 (REF. 63).

Figure courtesy of M. Duennwald, Boston Biomedical Research Institute, USA.

are a heterogeneous group, each affecting a distinct group of neurons and leading to distinct clinical phenotypes. With no clear relationship between expression levels and patterns of neuronal vulnerability or protein aggregation, these diseases epitomize the differential vulnerability of neuronal populations, a pervading and extremely perplexing feature of neurodegenerative diseases. It may seem unlikely that expression of a poly(Q)-expanded protein in a unicellular organism could give clues to differential neuronal

Box 3 | **A yeast model of Parkinson's disease and other synucleinopathies**



In the yeast strains shown in the figure, α-synuclein (α-syn)–green fluorescent protein (GFP) is expressed under a regulatable (galactose (Gal)-inducible) promoter at increasing levels of expression, resulting in no toxicity (NoTox), intermediate toxicity (IntTox) and high toxicity (HiTox). Immunofluorescence (left panels) shows that the localization shifts from the plasma membrane to intracytoplasmic aggregates with increasing expression. A spot assay (fivefold serial dilution starting with equal numbers of cells; right panel) shows decreased growth with higher levels of α-syn expression (the glucose 'off' control is not shown).

Genetic modifiers

- *Overexpression screen (intermediate toxicity wild-type α-syn strain).* 77 modifiers prominently represented vesicle trafficking, metal ion transport, osmolyte synthesis, protein phosphorylation, nitrosative stress and trehalose metabolism. One strong suppressor was the yeast homologue of *ATP13A2* (also known as *PARK9*) (see main text)[12,46].
- *Endoplasmic reticulum-to-Golgi trafficking.* Suppressors promoted and enhancers inhibited endoplasmic reticulum (ER)-to-Golgi anterograde transport — for example, Ypt1 (the homologue of mammalian RAB1), a GTPase that promotes the movement of vesicles from ER to the Golgi complex, suppressed toxicity, whereas Gyp8, the GTPase-activating protein that converts Ypt1 from its active GTP-bound state to its inactive GDP-bound state, enhanced toxicity. The modifier set suggested α-syn is likely to inhibit the docking or fusion of vesicles to the Golgi complex; this hypothesis was subsequently substantiated by ultrastructural studies and analysis in a cell-free system. Ultrastructural analysis showed that the large cytoplasmic α-syn inclusions seen with immunofluorescence are accumulations of undocked vesicles associated with α-syn[71,72]. Biochemical studies have shown 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 complex before degradation[12,46,71].
- *Trehalose pathway.* Three 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 organisms 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 non-toxic.
- *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 yeast metacaspase 1 (Mca1, also known as Yca1)[36]. The ResponseNet analysis (see main text) identified the genetic screen hit Gip2 as a novel conserved regulator of the heat-shock response[50].
- *Overexpression screen (mutant A30P α-syn).* Ypp1 was found to be a suppressor: when overexpressed it antagonizes reactive oxygen species accumulation and toxicity, binding to A30P α-syn and directing it towards degradation in the vacuole[73].
- *Synthetic lethality enhancer screen.* 32% of hits were related to lipid metabolism and vesicular transport[61].

Small-molecule screening

- *~10,000-compound library screen.* This screen identified flavonoids (including (–)-epigallocatechin 3-gallate (EGCG) and quercetin) as suppressors of α-syn toxicity at micromolar concentrations, and structure–activity relationship analysis implicated reduction in oxidative stress and metal chelation as possible mechanisms of action[74].
- *~115,000-compound library screen (commercial, natural product, NCI libraries).* Four 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[40]. These four compounds did not rescue growth of yeast cells expressing poly(Q)-expanded huntingtin exon 1 (Httex1), arguing for specificity.
- *Cyclic peptide screen.* From a pool of 5 million transformants, 2 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 seem to function downstream of the ER-to-Golgi trafficking defect. Structure–activity relationship data were rapidly generated for the peptides using point mutagenesis[41].

Validation in higher model systems

- *Impaired vesicle trafficking.* Multiple studies in neurons from mice lacking or overexpressing the *Snca* gene have identified a crucial role for α-syn in various stages of vesicle trafficking and in neurotransmitter release[75–77].
- *Validation of key genetic modifiers.* The human homologue of Ypt1, RAB1, suppressed α-syn toxicity in dopamine neurons of fruitflies, nematodes and mixed primary rat neuronal cultures[12]. In addition, RAB3A, which is highly expressed in neurons and localized to presynaptic termini, and RAB8A, which is localized to post-Golgi vesicles, suppressed toxicity in neuronal models of Parkinson's disease[71].
- *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[40,41]. The two leading compounds from the small-molecule screen also rescued rat dopamine neurons from toxicity induced by the mitochondrial toxin rotenone, strengthening the connection between α-syn and mitochondrial toxicity.

Figure courtesy of J. Valastyan and T. Outeiro, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts, USA.

vulnerability in a complex neurodegenerative disease. However, in yeast, poly(Q) toxicity is crucially dependent on subtle changes in the cellular proteome. Changes in the expression of just one other glutamine-rich protein can shift Httex1 from deadly to benign and vice versa. In some cases this seems to be due to the poly(Q)-expanded protein trapping and thereby titrating out an essential glutamine-rich protein[43]. In other cases, interaction with other non-essential glutamine-rich proteins can influence the conformational state of the Htt fragment[44]. A recent genome-wide suppressor screen supported this idea by showing that deletions of a number of glutamine- and asparagine-rich proteins suppressed toxicity[31]. In experiments that would have been impossible in any other system, the yeast model also revealed an exquisite dependence of poly(Q) 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, the poly(Q)-expanded Httex1 fragment only becomes toxic when Rnq1 is in the prion state[29]. The human proteome contains a large number of glutamine- and asparagine-rich proteins. Some of these might have the capacity to change their conformational status in a similar way. It seems likely that differential neuronal vulnerability is strongly influenced by changes in the expression and conformation of such proteins.

Studies in yeast have also revealed that the effect of the poly(Q) expansion is highly sensitive to flanking sequences. A recent study of 14 separate Httex1 constructs with varying sequences flanking the poly(Q) tract showed that a proline-rich region adjacent to the poly(Q) 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 poly(Q) expansion proteins differ profoundly in the amino acids flanking the poly(Q) region, the yeast data suggest that these flanking sequences are an important determinant of the unique toxicity and disease phenotype caused by each protein. Interestingly, the effect also works in *trans* because the enhanced toxicity conferred on poly(Q)-expanded Httex1 by the expression of other glutamine-rich proteins in the cell can be abrogated by the proline-rich region in Htt[45]. Such findings highlight the insights 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 overexpression screen of the yeast synucleinopathy model that included ~60 metal transporters, only 3 were recovered as toxicity modifiers and 2 of these were known to be involved in the transport of manganese[46]. The first transporter, Pmr1p, transports Mn^{2+} and Ca^{2+} ions from the cytoplasm into the Golgi complex 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[47]. The yeast interaction data set therefore 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 transporter family, and it strongly suppressed α-syn toxicity[46]. The human homologue 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*), were recently shown to cause early-onset PD (Kufor–Rakeb syndrome)[48]. Beyond its use 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 show that the deletion of *YOR291W* (also known as yeast *PARK9* (*YPK9*)) 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[49]. 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 available for yeast makes this model system an ideal candidate for analysing biological processes at a systems level. Cellular responses to stimuli have most commonly been assessed with transcriptional (mRNA) profiling and genetic screens. Earlier suggestions of a lack of overlap between these two types of high-throughput analysis were recently confirmed in yeast by comparing the transcriptional gene expression and genetic modifier data for 179 distinct perturbations[50]. 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 the cellular response. A computational method called 'ResponseNet' was devised to bridge gene expression and gene modifier data sets by using a yeast interactome data set relating 5,622 interacting proteins and 5,510 regulated genes through 57,955 protein–protein and protein–DNA interactions[50] (FIG. 2). The ResponseNet flow algorithm was designed to identify the most probable pathways connecting genes and proteins, therefore avoiding an unmanageable number of interactions. The ResponseNet network has succeeded in identifying components of well-studied pathways that were known from painstaking individual analyses in the past but that had eluded high-throughput genetic modifier and transcriptional data sets.

The ResponseNet algorithm was used to bridge high-throughput α-syn overexpression genetic modifier and microarray mRNA profile data sets. ResponseNet identified connections between pathways that were not prominently represented in either data set 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[36] (BOX 3). 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. These findings corroborated an earlier candidate-based analysis in yeast that implicated the heat-shock response in α-syn toxicity[36]. ResponseNet also 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, which are 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[51], and lovastatin enhanced α-syn toxicity in the yeast model. Together, these findings show that ResponseNet provides new avenues to mechanistically explore the basis of previously identified connections in human epidemiological studies, as well as to discover new gene–environment relationships.

Conclusions

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. Indeed, there are 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 using 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 data sets generated not only in yeast but also in other model systems and by human genome-wide analyses.

These achievements have certainly surpassed initial expectations. Beyond brewing of beer and leavening of bread, we envisage that, with the application of the latest molecular biology techniques, yeast is set to transform our understanding of complex human disease processes, including neurodegeneration.

Vikram Khurana is at the Departments of Neurology, Brigham and Women's and Massachusetts General Hospitals, Harvard Medical School, Boston, Massachusetts, USA; and at the Whitehead Institute for Biomedical Research, Cambridge, Massachusetts, USA.

Susan Lindquist is at the Whitehead Institute for Biomedical Research, Cambridge, Massachusetts, USA; the Department of Biology at the Massachusetts Institute of Technology, Cambridge, Massachusetts, USA; and is an Investigator of the Howard Hughes Medical Institute.

Correspondence to S.L.
e-mail: lindquist_admin@wi.mit.edu

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Competing interests statement

S.L. declares competing financial interests: see web version for details.

DATABASES

Entrez Gene: http://www.ncbi.nlm.nih.gov/gene
FXN | *YFH1*
UniProtKB: www.uniprot.org
α-syn | ATP13A2 | Bna4 | Ccc1p | HTT | Hsf1 | Hsp82 | Mca1 | Nde2 | Pmr1p | Rnq1 | Ypt1 | Yor291w

FURTHER INFORMATION

Susan Lindquist's homepage: http://web.wi.mit.edu/lindquist/pub
Addgene — S. *cerevisiae* Advanced Gateway Destination Vectors: www.addgene.org/yeast_gateway
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