Beer and Bread to Brains and Beyond: Can Yeast Cells Teach Us about Neurodegenerative Disease?

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Abstract

For millennia, humans have harnessed the astonishing power of yeast, producing such culinary masterpieces as bread, beer and wine. Therefore, in this new millennium, is it very farfetched to ask if we can also use yeast to unlock some of the modern day mysteries of human disease? Remarkably, these seemingly simple cells possess most of the same basic cellular machinery as the neurons in the brain. We and others have been using the baker's yeast, *Saccharomyces cerevisiae*, as a model system to study the mechanisms of devastating neurodegenerative diseases such as Parkinson's, Huntington's, Alzheimer's and amyotrophic lateral sclerosis. While very different in their pathophysiology, they are collectively referred to as protein-misfolding disorders because of the presence of misfolded and aggregated forms of various proteins in the brains of affected individuals. Using yeast genetics and the latest high-throughput screening technologies, we have identified some of the potential causes underpinning these disorders and discovered conserved genes that have proven effective in preventing neuron loss in animal models. Thus, these genes represent new potential drug targets. In this review, I highlight recent work investigating

mechanisms of cellular toxicity in a yeast Parkinson's disease model and discuss how similar approaches are being applied to additional neurodegenerative diseases.

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Introduction

As our population continues to age, neurodegeneration will increase in prevalence and thus pose a daunting challenge to public health worldwide. These truly disastrous neurodegenerative diseases include Alzheimer's, Huntington's, Parkinson's, amyotrophic lateral sclerosis and the frontal temporal dementias [1]. Interestingly, though disparate in their pathophysiology, many of these disorders share a common theme manifest in the accumulation of insoluble protein aggregates in the brain. One of the paramount research goals in the field is the elucidation of mechanisms causing these proteins to misfold and aggregate, as well as to understand their function in normal biology. Protein folding is critically important for all of life, from microbes to man [2]. A bafflingly diverse set of cellular mechanisms has evolved to coordinate this exquisitely sensitive process [3–7]. Since dealing with misfolded proteins is an ancient problem, some of the mechanisms employed to cope with them are likely conserved from yeast to man. Here I illustrate how basic studies in yeast cells can provide a toehold for ex-

ploring mechanisms of cell death attributed to the aberrant accumulation and/or function of human disease proteins.

Why Use Yeast?

Baker's yeast, or *Saccharomyces cerevisiae*, is a versatile experimental system for studying complex biological processes [8–12]. Notably, many of the key cellular pathways of yeast are very similar to those of mammalian cells and strains with gain- or loss-of-function mutations in these pathways are available [13, 14]. There are many advantages to the yeast system: the yeast genome is very well characterized and amenable to genetic manipulation [15, 16]. Methods are available to rapidly overexpress or knock out almost every gene and efforts to generate a collection of strains, each harboring a single mutation [17–19] as well as a collection of expression vectors with each yeast open reading frame [20–23] have recently come to fruition. Another major advantage of the yeast system is the availability of a rich and easily accessible dataset on genetic interactions, protein-protein interactions, transcriptional changes and protein localization [24–31]. Thus, the tools are now available and yeast is poised to be a robust new system for investigating, on a genome-wide scale, the mechanisms underlying many cellular processes, with direct relevance to human disease and for the discovery of novel drug targets for therapeutic intervention [32–37]. While at first it may seem implausible that yeast cells will provide any insight into mechanisms of neurodegenerative disease, it is worth noting that almost everything we know today about cancer biology has, as its foundation, basic studies begun in yeast [38, 39].

Parkinson's Disease and α -Synuclein

Parkinson's disease (PD) is the second most common neurodegenerative disorder, exceeded by only Alzheimer's disease [40–47]. Clinical manifestations of PD include severe motor defects characterized by resting muscle tremor ('pill-rolling'), muscle rigidity, bradykinesia and postural instability [48]. PD is characterized by the selective loss of dopaminergic neurons from substantia nigra pars compacta [49]. Accumulating evidence points to a causative role for the presynaptic protein α -synuclein (α -syn) in the pathogenesis of PD. α -Syn is a major constituent of Lewy bodies, cellular inclusions that are the pathological hallmark of PD as well as several other neurode-

generative disorders [50–53]. Moreover, several missense mutations in the α -syn gene (A53T, A30P, E46K) [54–56] as well as duplication or triplication of the wild-type locus have been shown to cause PD [57–59].

Genetically tractable model organisms have been employed to study the role of α -syn in PD [60–62]. Overexpression studies of α -syn, or its PD-linked mutants, in mouse, rat, fly and nematode have all converged on the conclusion that increased levels of α -syn lead to neurotoxicity [63–68]. But despite intense study of α -syn, frustratingly little is known about its normal cellular function and how that function contributes to the disease [69, 70]. Yet it is becoming increasingly clear that, for a number of disease proteins, key to disease may be understanding the normal function of the protein [71, 72].

Yeast Model of PD

Protein misfolding and aggregation can be deleterious to all cell types. Reasoning that α -syn accumulation might pose a similar problem to yeast cells as it does to neurons, and that it therefore represents a particularly tractable platform to investigate the cell biology of these defects, Outeiro and Lindquist [34] created a yeast model of α -syn cellular toxicity. They began by constructing a yeast strain that expressed α -syn fused to the green fluorescent protein (GFP) to allow in vivo visualization of the protein. This fusion protein localized strongly to the plasma membrane (fig. 1a), consistent with the propensity of α -syn to interact with phospholipids [73–80]. Remarkably, simply doubling the expression levels of α -syn dramatically changed its localization, with the majority of α -syn now found in large cytoplasmic inclusions (fig. 1b). Moreover, whereas a single copy of α -syn had no significant effect on cell growth, 2 copies resulted in profound cytotoxicity, resulting in growth inhibition and cell death (fig. 1c). Thus, expressing α -syn in yeast provided a simple, yet powerful model system for systematically interrogating the cellular consequences of aberrant α -syn accumulation [21, 79, 81–85].

A Genome-Wide Screen in Yeast Identifies Modifiers of α -syn Toxicity

We recently carried out a genome-wide screen in yeast to discover suppressors and enhancers of α -syn cytotoxicity [21; unpubl. data]. We reasoned that the types of modifier genes identified in our screen would provide in-

Fig. 1. Expressing α -syn in yeast. **a** A single copy of α -syn-GFP is localized to the plasma membrane. **b** When a second copy is integrated into the genome, doubling its accumulation, the fate of the protein is profoundly altered; the vast majority of α -syn-GFP appears in large cytoplasmic inclusions. **c** One copy of α -syn has little or no effect on growth, while 2 copies show complete growth inhibition.

sight into the specific cellular pathways perturbed by α -syn accumulation, and ultimately suggest novel avenues for therapeutic investigation. A critical aspect of high-throughput screening is the reliability of the starting library and the harmonization of screening procedures. Accordingly, we participated in efforts to generate what will become the 'gold standard' in yeast genomic libraries. In collaboration with the Harvard Institute of Proteomics (Cambridge, Mass., USA), we helped create an arrayed library of 5,000 completely sequence-verified yeast genes in Gateway[®] cloning system-based expression vectors [20, 21]. Unlike existing libraries that contain fragments of genes and/or often mutations that complicate interpretation of results, this library contains only full-length genes and is free of mutations.

Using this library, we performed a high-throughput yeast overexpression screen to identify modifier genes that antagonized cellular toxicity resulting from the ac-

cumulation of the PD-linked α -syn protein [21; unpubl. data]. As illustrated in figure 2, each of the 5,000 genes was introduced into a yeast strain that also expressed α -syn and the ability of each gene to ameliorate or worsen the toxicity was assessed. Intriguingly, many of the yeast genes identified that had effects have clear human orthologues. The largest and most effective class of modifiers was a group of highly conserved genes involved in vesicle-mediated transport between the endoplasmic reticulum and Golgi. Moreover, the earliest defects in yeast cells accumulating α -syn was impaired endoplasmic reticulum-Golgi trafficking [21].

We sought to extend and validate these findings from yeast in neuronal PD models. In collaboration with Drs. Nancy Bonini (University of Pennsylvania; fruit fly PD model), Guy Caldwell (University of Alabama; worm PD model) and Jean-Christophe Rochet (Purdue University; rat embryonic neuron culture model), we tested the abil-

Fig. 2. Genome-wide α -syn toxicity modifier screen. Yeast cells poised to express α -syn were transformed individually with each of $\sim 5,000$ expression plasmids, each harboring a unique full-length yeast open reading frame (ORF). Transformants were selected by growth in media lacking uracil and then spotted onto agar plates containing galactose, to induce expression of α -syn and the gene of interest. Plates were incubated for 2–3 days and genes that suppressed or enhanced α -syn toxicity were identified by those that modified the ability of the yeast to grow: Suppressors allow more robust growth, while enhancers knock down the ability to grow even further than with α -syn alone.

ity of Rab1, the mammalian orthologue of Ypt1p – one of our best yeast α -syn toxicity suppressors, to prevent dopaminergic neuron loss. Remarkably, in each model tested Rab1 coexpression was sufficient to suppress α -syn-induced dopaminergic neuron loss. Additional work is required to delineate the mechanisms by which Rab1 antagonizes α -syn toxicity; however, these results serve as proof-of-concept that hits uncovered from our yeast screen can have direct relevance to neuronal α -syn pathophysiology. Importantly, this paradigm of yeast high-throughput screening followed by validation in animal models of PD is readily amenable to small-molecule drug screens for PD therapeutics.

Rising to the Occasion: Additional Yeast Models of Neurodegenerative Disease

Huntington's Disease

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder characterized by the progressive loss of medium spiny neurons from the striatum and cerebral cortex, causing involuntary movements, speech difficulties, problems with balance and swallowing, depression, mood swings, dementia, and inevitably death [86, 87]. The HD gene was defined in 1993, and encodes an enormous protein called huntingtin, whose normal function still remains enigmatic [88]. The N-terminal region of huntingtin contains a polyglutamine (polyQ) tract and HD patients harbor pathogenic polyQ

expansions [89–91]. Postmortem evaluation reveals cytoplasmic and/or intranuclear inclusions of aggregated fragments of expanded polyQ huntingtin protein in the brains of affected individuals [87, 92]. Though variable, all healthy individuals have less than 37 glutamines and those with more than 40 are certain to develop HD [93]. Moreover, the greater the size of the polyQ expansion the earlier the age of onset of the disease [89].

Several groups have used yeast to explore the cellular consequences of polyQ expansions [94–97]. Intriguingly, expressing a fragment of huntingtin fused to GFP results in polyQ length-dependent aggregation; fusion proteins containing nonpathogenic Q lengths of 25 or fewer are diffusely distributed throughout the cytosol, whereas Q lengths of 72 and 103 result in the formation of tight fluorescent foci. In certain contexts, expressing these proteins in yeast cells also results in Q length-dependent cytotoxicity [95]. Thus, the yeast model faithfully recapitulates 2 key features of the disease: Q length-dependent aggregation and cytotoxicity. Recent work in yeast has provided insight into the contributions of amino acid sequences flanking the polyQ region within huntingtin that modulate its aggregation and toxicity [98]. No other model system is as amenable to this type of rapid *in vivo* structure/function analysis as yeast.

Genome-wide screens in yeast have been performed to identify modifiers of huntingtin aggregation and cellular toxicity [99, 100]. A synthetic lethal screen, in which a partially toxic huntingtin construct was introduced into each of 4,850 nonessential gene deletion strains to iden-

tify toxicity enhancers, uncovered genes involved in protein folding, response to stress and ubiquitin-mediated protein degradation [100]. Interestingly, a similar screen with PD-linked α -syn resulted in a nonoverlapping set of genes, suggesting that the phenotypes in yeast were not merely attributed to nonspecific effects of overexpressing a misfolded foreign protein, but rather were exquisitely specific to the underlying biology of the individual disease protein. A complementary screen was also performed looking for gene deletions that suppressed the toxicity of polyQ huntingtin protein [99]. Undoubtedly, large-scale small molecule screens are underway to identify potential HD therapeutics [101]. As one example, green tea polyphenol (–)-epigallocatechin-3-gallate (EGCG) has already shown promise in ameliorating polyQ toxicity and aggregation in yeast as well as in animal models of HD [102]. The yeast platform will be critical for refining and improving the efficacy of such lead compounds. Finally, it is noteworthy that there are at least 9 inherited neurodegenerative diseases, including HD, caused by polyQ expansions [103]. It is likely that studies in the yeast polyQ model will provide insight into mechanisms of these other related diseases as well.

Friedreich's Ataxia

Friedreich's ataxia (FRDA) is an autosomal recessive degenerative disorder primarily affecting the nervous system and heart. Clinical features of FRDA include ataxia, sensory loss, muscle weakness and cardiomyopathy [104]. In 1996, the gene responsible for FRDA was cloned and found to encode a relatively small protein, which was termed frataxin [105]. Unfortunately, frataxin's primary amino acid sequence did not shed light on its potential cellular function. But it was noted that there were orthologues present in worm and yeast. By 1997, 2 groups had seized on this observation and generated yeast models of FRDA [106, 107]. The yeast gene *YFH1* (yeast frataxin homologue) was originally identified by virtue of its ability to suppress the phenotype of a mutant strain unable to grow on iron-limited medium, suggesting a potential role for frataxin in iron metabolism [106]. Yeast cells lacking *YFH1* exhibited profound mitochondrial defects; *yfh1Δ* cells are unable to grow on nonfermentable carbon sources (indicating respiratory deficiency) and, in some strain backgrounds, completely lacked mitochondrial DNA [106, 107]. In further support for a role in mitochondrial function, Yfh1p is localized to mitochondria. Remarkably, wild-type human frataxin, but not a patient-derived point mutant, is sufficient to rescue the growth phenotype of *yfh1Δ* cells. Clinical and

pathological overlap between FRDA and mitochondrial diseases had long been appreciated [108], but these groundbreaking studies in yeast provided the first direct link between FRDA, frataxin, iron metabolism and mitochondrial function.

Subsequent studies have indicated a role for frataxin in the assembly and/or maintenance of iron-sulfur clusters, which are prosthetic groups in many mitochondrial proteins, including aconitase and the first 3 respiratory complexes [109, 110]. The yeast system is well suited to help answer some of the immediate questions that these findings raise: which proteins interact with frataxin/Yfh1p? Which genes are able to modify the *yfh1Δ* growth phenotype and thus help to further delineate the cellular functions for frataxin/Yfh1p? And perhaps most attractive about this yeast FRDA model will be the ability to perform high-throughput drug screens to identify potential FRDA therapeutics.

Niemann-Pick Disease Type C

Niemann-Pick disease refers to a collection of lysosomal storage disorders, including types A, B and C. Niemann-Pick disease type C (NP-C) is a fatal autosomal recessive neurodegenerative disease characterized by abnormal accumulation of low-density lipoprotein-derived cholesterol [111]. Though clinically heterogeneous, the most common forms of NP-C begin in the late-infantile and juvenile periods. Clinical manifestations include hepatic, neurologic and psychiatric phenotypes. Later symptoms include movement and speech difficulties [112, 113]. Positional cloning of the NP-C gene (*NPC1*) revealed a closely related gene present in yeast (*NCR1*) [114]. Indeed, expressing the yeast Ncr1p protein in *NPC1*-deficient mammalian cells is sufficient to reverse cholesterol accumulation defects, suggesting a conserved function between the yeast and human proteins [115]. Introducing dominant mutations into Ncr1p results in defects in sphingolipid metabolism, but deleting the yeast *NCR1* gene has no effect on cell growth under basal conditions [115]. However, *ncr1Δ* cells are not completely normal; they exhibited a dramatic resistance to the antitumor ether-lipid, alkylphosphocholine drug, edelfosine [116]. This effect is completely eliminated when the wild-type *NCR1* gene is reintroduced, strongly suggesting that this drug resistance phenotype is attributed to loss of *NCR1*. Thus, the yeast NP-C model system provides a tractable platform for performing structure/function analysis to determine the effect of specific patient mutations on the *NPC1*/Ncr1p protein as well as small-molecule and genetic screens to discover possible treatments for NP-C.

Fig. 3. Using the yeast models to explore mechanisms of neurodegeneration and for discovering genetic and small molecule modifiers. Each new yeast model of neurodegenerative disease can be integrated into a pipeline of genetic screens (synthetic lethal screen and overexpression screen), transcriptional profiling by microarray, and high-throughput small-molecule screens. Measured readouts can be toxicity, aggregation or other more specialized phenotypes.

Prion Diseases

The transmissible spongiform encephalopathies are a collection of fatal neurodegenerative disorders, including bovine spongiform encephalopathy ('mad cow disease') in cattle, scrapie in sheep, as well as Creutzfeldt-Jakob disease, Gerstmann-Sträussler syndrome, kuru, and fatal familial insomnia in people [117–120]. Humans with prion disease present with dementia and, in some cases, cerebellar ataxia. Postmortem pathological examination reveals spongiform degeneration and astrogliosis in the brains of afflicted individuals [121]. Overwhelming evidence implicates the prion protein PrP as the causative agent in transmissible spongiform encephalopathies [122–124]. PrP exists in 2 conformations, designated PrP^C (normal form) and PrP^{Sc} (disease form). The protein-only prion hypothesis posits PrP^{Sc} is itself the infectious agent because it templates the conversion of existing PrP^C to the PrP^{Sc} form, thus creating a vicious cycle of infectivity [125, 126]. Little is known about PrP's cellular function (mouse PrP knockout mice are grossly normal [127, 128]), though emerging evidence suggests it might play a role in stem cell maintenance and neurogenesis [129, 130]. The yeast system has been used to study the conversion of PrP^C to PrP^{Sc} [131]. Expression of PrP in the yeast cytoplasm resulted in robust conversion of PrP^C to a protease-resistant and detergent-insoluble PrP^{Sc}-like conformation [131]. The yeast system is the perfect venue for performing large-scale genetic and chemical screens to look for

modifiers of PrP^C → PrP^{Sc} conversion. Yeast has also been used to investigate the normal cellular function of PrP [132]. When expressed in yeast cells, the mammalian proapoptotic protein Bax causes cell death. Similar to what had been observed in neurons, coexpressing PrP was sufficient to rescue this lethality, providing evidence for a cytoprotective role of PrP. Yeast screens could be used to identify genes and small molecules that modulate PrP activity.

The Effect of Aging on Yeast Neurodegenerative Disease Models

Aging is by far the most common risk factor for developing neurodegenerative disease [133]. Strikingly, many of the key aspects of yeast aging closely resemble those of mammalian cells, especially postmitotic neurons (for example oxidative stress, decreased proteasome function and the accumulation of somatic mutations) [134–136]. The biology of postmitotic cells is profoundly different than that of dividing cells and since in almost all of these diseases it is predominantly postmitotic neurons that are affected, examining yeast models of neurodegenerative disease in the context of nondividing aged cells will be crucial, and perhaps much more directly relevant to the biology of aging neurons [137].

There are 2 types of aging models in yeast: replicative and chronological [138, 139]. Replicative aging refers to the number of cell divisions a mother cell will undergo before dying (~20 divisions), whereas chronological aging refers to the ability of yeast cells, when nutrients are depleted, to exit the cell cycle and remain viable in stationary phase for weeks [137]. Yeast cells in stationary phase must cope with the same challenges that the neurons in our brain face over a lifetime: the accumulation of damaged proteins, oxidative stress, proteasome impairment and the buildup of mutations, without the ability to dilute out these cellular insults by dividing. It will be important to test the effect of stationary phase aging on existing as well as future yeast models of neurodegenerative disease [140]. Finally, the expanding repertoire of yeast genomic resources will facilitate high-throughput genetic screens to elucidate key regulators of aging, leading to therapeutic strategies aimed at boosting the ability of neurons to deal with the unrelenting cellular stresses associated with the aging process [141].

Create Your Own Yeast Disease Model

The above examples illustrate the many different ways yeast cells have been applied to studying neurodegenerative diseases. Yet, this approach is simply the tip of an iceberg, providing the foundation for many additional

yeast models for neurodegenerative and other diseases. In some cases, heterologous expression of a wild-type or mutant human protein will be necessary, while in other instances, it may be of interest to mutate the yeast homologue of a human disease gene. Creating fusions to fluorescent proteins (for example GFP and DsRed) or protein tags (for example FLAG, HA and TAP) will enable the visualization of aggregate formation and dynamics as well as the purification of protein complexes containing the disease protein of interest. For protein-misfolding diseases, yeast can be used to monitor the aggregation properties of a disease protein (for example Alzheimer's amyloid β peptides) and might bring a fresh perspective to the debate over what truly constitutes the toxic species in many of these disorders [142]. Newly developed yeast models could then be rapidly plugged into a multifaceted regime of genetic and small-molecule screens (fig. 3) to discover novel modifiers of aggregation and toxicity as well as to help assign cellular functions to new disease genes.

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