Modeling ALS and FTLN proteinopathies in yeast

An efficient approach for studying protein aggregation and toxicity

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Key words: amyotrophic lateral sclerosis, ALS, frontotemporal lobar degeneration, FTLN, yeast, amyloid, prion, FUS, TDP-43, SOD1

Abbreviations: FUS, fused in sarcoma; TDP-43, TAR DNA binding protein 43; SOD1, super oxide dismutase 1; ALS, amyotrophic lateral sclerosis; FTLN, frontotemporal lobar degeneration; PD, Parkinson disease; HD, Huntington disease; HTS, high-throughput screening

In recent years there have been several reports of human neurodegenerative diseases that involve protein misfolding being modeled in the yeast *Saccharomyces cerevisiae*. This review summarizes recent advances in understanding the specific mechanisms underlying intracellular neuronal pathology during Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Lobar Degeneration (FTLN), including SOD1, TDP-43 and FUS protein inclusions and the potential of these proteins to be involved in pathogenic prion-like mechanisms. More specifically, we focus on findings from yeast systems that offer tremendous possibilities for screening for genetic and chemical modifiers of disease-related proteotoxicity.

Overview of ALS and FTLN

Many progressive neurodegenerative diseases are associated with the intra- or intercellular aggregation of specific proteins in neurons. For these proteinopathy disorders, the precise pathological role of protein aggregation in cellular degeneration is not clearly understood, yet particular disorders are tightly linked to very specific protein aggregation and histological patterns of neuronal death. Two neurodegenerative diseases, amyotrophic lateral sclerosis and frontotemporal lobar degeneration, which have partially overlapping clinical presentations,^{1,2} have recently been identified as sharing common features of abnormal protein accumulation.^{3,4}

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder that generally strikes in late midlife, with death usually occurring from respiratory failure within 5 years of initial symptom onset. The pathological hallmark of ALS is the selective loss of upper and lower motor neurons, but the specific underlying

causes remain unknown. Approximately 2–3 of 100,000 people per year are diagnosed with ALS,^{5,6} with most cases characterized as being sporadic in origin, but approximately 10% are considered inherited because of a familial history. Thus far, several genes are linked to various forms of ALS. Mutations in the genes *SOD1*, *TARDBP* and *FUS* (also known as *TLS*) are considered causative for familial ALS and represent the largest percentage of identified familial disease genes. Furthermore, the protein products of these genes—SOD1, FUS and TDP-43 (product of *TARDBP* gene)—are each known to accumulate independently as cytoplasmic inclusions in various forms of the disease.

Frontotemporal lobar degeneration (FTLN) is an umbrella term that encompasses several clinically similar syndromes (reviewed in ref. 7), including frontotemporal dementia (FTD), semantic dementia (SD), progressive non-fluent aphasia (PNFA) and FTD with motor neuron disease (FTD/MND). FTD alone is a leading cause of progressive dementia, with a prevalence as high as 15 per 100,000,⁸ and is clinically distinguished from Alzheimer disease by distinct early behavioral changes prior to full onset dementia. The FTLN syndromes are united pathologically by degeneration of the anterior cerebral hemispheres. The distribution of tissue atrophy is variable on the frontotemporal lobes, and is generally linked to disease subtype manifestation.⁹ FTLN-causing mutations have been found in several genes, including *microtubule-associated protein Tau* (*MAPT*),¹⁰ *progranulin* (*GRN*),^{11,12} and *charged multivesicular body protein 2B* (*CHMP2B*).¹³ Three major subtypes of FTLN are respectively linked to neuronal inclusions containing type-specific proteins, such as TDP-43, Tau and FUS.¹⁴ TDP-43 subtype represents more than 50% of FTLN cases and is rarely associated with mutations in the *TARDBP* gene.^{15–17}

The cytoplasmic accumulation of the proteins TDP-43 or FUS, and their link to familial and sporadic forms of ALS and FTLN, demonstrates the existence of common underlying mechanisms in these diseases.^{3,18–22} Identification of TDP-43 and FUS as biomarkers in most cases of ALS and FTLN opens a new avenue for researchers. Given that there are no long-term effective treatments for ALS or FTLN and that the

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Submitted: 07/01/11; Accepted: 07/29/11
DOI: 10.4161/pri.5.4.17229

Figure 1. Structural organization of ALS-associated proteins SOD1, TDP-43 and FUS. SOD1 is rich in β -sheet secondary structure, indicated by blue arrows. Copper-coordinating histidine residues are indicated with red circles (amino acids 46, 48, 63 and 120) and zinc-coordinating histidine residues are indicated with green circles (amino acids 63, 71, 80 and 83). TDP-43 and FUS share similar structural elements, including RNA-recognition motifs (RRM) and glycine-rich regions. The protein segments that are sufficient for yeast cell growth inhibition by overexpression are bracketed in red. FUS possesses a large amino-terminal tract that resembles yeast prion domains and is rich in the amino acids serine, glutamine, glycine and tyrosine (SQGY). It also possesses arginine/glycine-rich (R/G-rich) domains and a zinc finger (ZF) domain. The carboxy-terminal nuclear localization signal (NLS) of FUS is the site of several ALS-linked mutations.

causes of protein accumulation and toxicity are unresolved, there is a pressing need to develop models to target intracellular protein inclusions for future therapy. In recent years several *Saccharomyces cerevisiae* yeast models have been developed to address this issue and have proven effective for our understanding of basic pathological mechanisms of protein misfolding diseases.

The Yeast Model System for Studying Human Neurodegenerative Diseases

The yeast *Saccharomyces cerevisiae* has been an undisputedly productive model organism for elucidating fundamental eukaryotic cellular mechanisms. Its significance is that it is not only famously tractable, but at the cellular level, key processes are conserved between yeast and higher eukaryotes, including humans. Nearly a third of yeast genes have a direct human ortholog,²³ and as many as two thirds have domains with significant homology to human genes.²⁴ Importantly, there are currently about 500 human disease genes (~22%) that are orthologous to yeast genes,²⁵ and for this reason yeast is also an excellent eukaryotic surrogate for studying human disease.

The yeast model system is also increasingly being used to model neurodegenerative diseases,²⁶⁻²⁹ many of which are linked to the misfolding and aggregation of specific proteins. It is thought that the accumulation and aggregation of these proteins is disruptive to critical cellular pathways that are integral to proper functioning of neuronal cells. The experimental approach to modeling these diseases in yeast generally involves the overexpression of a specific disease-related protein and monitoring how its aggregation and localization disturb cellular processes and retard cell viability. Obviously yeast do not recapitulate the specialized cell-cell interaction networks in neural tissue, but due to the highly conserved eukaryotic machinery, such as protein

quality-control systems, the intracellular mechanistic upheavals observed in yeast are believed to model similar problems in diseased neurons.

Parkinson disease (PD), Huntington disease (HD) and Alzheimer disease have each been modeled to various degrees in yeast (reviewed in ref. 26, 28 and 29). In particular, the aggregation and toxicity of α -synuclein and polyglutamine (polyQ)-expanded Huntingtin (associated respectively with PD and HD) have been very thoroughly studied in yeast and both proteins show different patterns of aggregation with distinctive mechanisms of toxicity. In the case of α -synuclein, as its expression levels are increased, the protein transitions from being localized to the plasma membrane to forming cytoplasmic

aggregates, which also coincides with growth inhibition and cell death. This toxicity recapitulates neuronal cellular pathology, including defects in vesicle trafficking, proteasomal degradation and mitochondrial function.³⁰⁻³³ When polyQ-expanded Huntingtin fragments are overexpressed in yeast, they form toxic cytoplasmic aggregates in a length-dependent fashion that are disruptive to ER-associated degradation, endocytosis and mitochondrial function.³⁴⁻³⁶ Expression of human Tau in yeast enabled detection of hyperphosphorylated, sarkosyl-insoluble Tau fractions, similar to those found in patients with Alzheimer disease.³⁷ After isolation from yeast, this fraction efficiently seeded recombinant Tau fibrilization in vitro; moreover, deletion of yeast kinases Mds1 and Pho85 (orthologs of human GSK-3 β and cdk5) modulated Tau phosphorylation and aggregation, mimicking processes that occur in diseased tissue.

The value of using yeast for modeling cellular neurodegenerative processes is validated by the fact that results in yeast can be reproduced in higher organism models like flies and mice. For example, protein homeostasis dysfunction in the ER resulting from polyQ expression in yeast³⁴ was recapitulated in both a mouse model and human disease tissue.³⁸ Similarly, human proteins that can modify α -synuclein or TDP-43 proteotoxicity in animal models were first identified through genetic assays using yeast models (discussed below).

Modeling ALS/FTLD Proteinopathy in Yeast

SOD1 models. For many years the only known ALS-linked gene was *SOD1*,³⁹ which encodes Cu, Zn superoxide dismutase (SOD), a conserved cytosolic protein implicated in the defense against free radicals (Fig. 1). The primary known cellular function of SOD1 is the detoxification of superoxide radicals ($O_2^{\cdot-}$), although several additional activities have also been proposed.⁴⁰ Currently, more than 140 different ALS-associated mutations

have been identified in *SOD1*, accounting for about 20% of familial ALS cases. Most intriguingly, these mutations are scattered throughout the *SOD1* sequence and likely affect different aspects of the protein's function (or structure), such as metal binding affinity or enzymatic activity. Many mutant proteins retain full SOD activity in vitro and in vivo, and are thought to exert their toxic effects by a gain-of-function mechanism. Most likely, this toxic mechanism is linked to the increased aggregation propensity of *SOD1* mutants,⁴¹ yet still no accepted theory exists to explain how so many different mutations can cause a common manifestation of disease.

Initial studies of *SOD1* in yeast were to determine the functional activity of *SOD1* mutants in vivo. Substitution of the yeast *SOD1* ortholog (54% amino acid identity) with either wild type or mutant human *SOD1* resulted in full phenotypic complementation of the deleted yeast *SOD1* gene, such as the elimination of sensitivity to hyperoxia and redox cycling drugs.⁴² This provided early evidence that many *SOD1* mutants can retain normal functional activity, which was further supported in studies of transgenic mice overexpressing human *SOD1* mutants.⁴³⁻⁴⁵ The yeast model has also provided useful knowledge about the relative copper-binding affinities of the ALS-linked *SOD1* mutants in vivo, by measuring SOD activity of mutants in crude lysates and analyzing the ability to complement *sod1Δ* phenotypes.⁴⁶ Also, the copper chaperone, CCS (Lys7 in yeast), which is necessary for the *SOD1* copper binding ability in vivo, was discovered in yeast.⁴⁷ More recently, specific targeting of *SOD1* into the yeast mitochondrial intermembrane space was shown to be protective against respiration derived oxidative stress, arguing for the functional relevance of the presence of *SOD1* in this compartment.^{48,49}

TDP-43 and FUS models. Until 2006, accumulation of misfolded *SOD1* in motor neurons was the dominant theory for the pathogenesis of familial ALS and even sporadic ALS. The situation changed dramatically with the discovery of two novel ALS-associated proteins, TDP-43 and FUS. Both proteins have been found in cytoplasmic inclusions in motor neurons and glial cells of ALS and FTLN patients (reviewed in ref. 50), and mutations in the genes coding these proteins are causative for familial forms of ALS.⁵¹⁻⁵⁴ At first glance, TDP-43 and FUS share similar structural organization (Fig. 1) and may have similar functions connected with RNA processing. They contain RNA-recognition motifs as well as glycine-rich regions involved in RNA and protein interactions. Both are predominantly nuclear proteins that play a role in RNA trafficking and can shuttle between the nucleus and cytoplasm.^{55,56} Disease conditions are coupled with cytoplasmic accumulation and aggregation for both proteins, which may be induced by mutations. For example, most TDP-43 mutations, as well as about a third of FUS mutations, are concentrated within the large glycine-rich region⁵⁷ and may promote processes such as misfolding, mislocalization or formation of aberrant molecular complexes.

Several recent attempts to model TDP-43 and FUS proteinopathy in yeast have helped dissect the aggregation propensity and toxicity associated with different protein domains (Fig. 1B and C), as well as the effects of various disease-associated mutations.⁵⁸⁻⁶³ Unlike *SOD1*, these proteins do not have

Figure 2. Modeling FUS and TDP-43 proteotoxicity in yeast. Protein aggregation and inhibition of growth enable yeast disease models to be used for high-throughput genetic and chemical screens for factors that ameliorate toxicity and/or aggregation. Fusion with green fluorescent protein (GFP) allows for the easy visualization of protein aggregates and has no observable effect on proteotoxicity. (A) Overexpression of the ALS/FTLD-linked proteins FUS and TDP-43 results in severe inhibition of yeast cell growth. The strains are serially diluted and grown on medium (in this case, galactose medium) that induces high expression of the desired protein. (B) The aggregation of FUS and TDP-43 can be monitored by fluorescent microscopy using GFP fusions. (C) Co-expression of a nuclear-localized marker (NLS-tomato) with FUS-GFP indicates that FUS forms predominately cytoplasmic aggregates (Figure part adapted from ref. 62).

clear homologs in yeast. Both TDP-43 and FUS were expressed in yeast as fusions with green fluorescent protein (GFP) to monitor localization and their predisposition to aggregation (Fig. 2). Importantly, such GFP fusions do not interfere with the aggregation and toxicity associated with the induction of TDP-43 and FUS expression.

The first yeast model of these proteinopathies was established with the overexpression of TDP-43.⁵⁹ At low expression levels, TDP-43 partitions to the yeast nucleus, as it would in healthy neurons, likely due to the function of the nuclear localization signal (NLS) located in its amino terminus. At higher expression levels, or with the removal of its amino-terminal domain, TDP-43 accumulates in the yeast cytoplasm and forms sub-cellular aggregates that are tightly linked to cell growth inhibition and cytotoxicity,⁵⁹ recapitulating its behavior in cell culture models. Expression of different truncations of TDP-43 indicated that toxicity potential resides in the second half of the protein (Fig. 1), which contains one RNA-recognition motif and a glycine-rich domain.

The yeast TDP-43 proteinopathy model was also used to suggest that TDP-43 aggregates introduce a gain-of-function toxic

species.⁶⁰ Specific TDP-43 mutations associated with familial ALS were introduced in the yeast model and their effect on aggregation and toxicity was compared with wild-type TDP-43. Some mutations resulted in greater and more rapid protein aggregation tendency and this correlated with greater cytotoxicity. Such mutations were also confirmed to result in larger potential for aggregation in vitro, supporting the idea that mutations that promote the misfolding of TDP-43 are causative for motor neuron degeneration.⁶⁰

FUS proteinopathy yeast models have likewise been developed that follow much of the same logic employed with the TDP-43 models.^{58,61–63} Unlike TDP-43, nearly the entire FUS protein except for a segment of the carboxy-terminal domain must be expressed to induce cytotoxicity (Fig. 1). FUS also has a strong propensity for forming cytoplasmic inclusions in yeast cells (Fig. 2), even disparate sub-domains that exhibit no individual toxicity were shown to form cytoplasmic foci following over-expression. However, full-length (or nearly full-length) FUS appears to have the greatest proclivity for aggregation, which is associated with a concomitant cytotoxicity.

ALS-associated mutations in the most carboxy-terminal region of FUS were introduced in the yeast model.⁶¹ In contrast with some TDP-43 mutations, there were no significant effects on FUS toxicity and aggregation. This may be because mutations of FUS that are localized to the extreme carboxy-terminal domain are believed to result in the aberrant partitioning of FUS in neurons to the cytoplasm because they are disruptive to the FUS NLS.^{64–67} The wild-type sequence of this NLS was shown to not function well in yeast,⁶¹ thus disruptive mutations would have little impact in the yeast model. This perhaps illustrates the strength of using wild-type FUS to effectively model cytotoxicity in yeast because even at low expression levels FUS can accumulate in the cytoplasm, mimicking the effects of various FUS mutants that fail to properly localize to the nucleus. Overall, FUS yeast models are able to recapitulate aggregation and cytotoxicity associated with impairment of normal nuclear import of FUS, arguing for a toxic gain-of-function mechanism for FUS proteinopathies.

Do FUS, TDP-43 and SOD1 Propagate Like Prions?

When FUS was first identified as being linked to familial ALS, the amino-terminal domain of the protein was cause for speculation because its first approximately 165 amino acids (Fig. 1B) are very similar in composition to the prion domains found in most yeast prion proteins.^{68,69} It was theorized that a prion-like behavior of this domain could be responsible for the spread of FUS-linked pathology in motor neurons. Yeast prion domains are generally large protein segments highly enriched in hydrophilic amino acids, such as glutamine and asparagine, with a paucity of charged residues.^{70,71} These domains confer the capacity of a protein to be a prion (an infectious protein), which is a protein that can stably maintain either of two conformational states: a 'normal' state and an infectious (usually aggregated) prion state. Typically, the aggregated infectious state is an amyloid, which is a highly-ordered filamentous protein polymer with

cross- β architecture, meaning that it is rich in β -sheet secondary structure and the β strands are generally aligned perpendicular to the long axis of the filament. In their amyloid form, proteins usually have increased protease and detergent resistance, and they show specific staining with the dyes Congo Red and thioflavin T. Amyloid-based prions are distinguished from other amyloids by increased ability to self-propagate by causing non-prion molecules of the same protein to be incorporated into the aggregate.⁷² This auto-catalytic process, together with increased resistance to proteases and destabilizing agents, enables prions to be infectious through cell-to-cell and organism-to-organism transmission. It is an intriguing possibility that ALS-associated proteins could have such a prion-like pathogenic mechanism.

A simple genetic assay to test if a particular protein segment has the capacity to propagate as a prion in yeast is to fuse the segment to a reporter protein, such as Sup35C—a functional part of Sup35 prion protein lacking its native prion domain (reviewed in ref. 73–76). Our group fused various amino-terminal segments of FUS to Sup35C and found no evidence that this domain conferred prion capacity in yeast.⁶² Interestingly, aggregation propensity of the FUS 1–183 aa fragment was much lower compared to full-length FUS protein. It may reflect the lower percentage of asparagines in FUS 1–183 aa (~3%) compared to the prion domains of Sup35 (~16%), Ure2 (~36%) and Rnq1 (~17%), the most-studied yeast prions. Despite chemical similarity, glutamines and asparagines are surprisingly different in their ability to promote self-templating amyloids.⁷⁷ Thus, despite the presence of significant amount of glutamines, insufficient asparagine content might impair prion-forming ability of the FUS N-terminal domain. Another possibility is the almost complete lack of hydrophobic residues in the FUS amino terminus compared to these prion domains. Hydrophobic residues are believed to be a contributing force in prion propensity by participating in intermolecular hydrophobic interactions,⁷¹ and perhaps also as recognition elements for molecular chaperones that facilitate prion propagation.^{78,79} Thus, while there is superficial similarity between the FUS N-terminal domain and yeast prion domains, it is not necessarily extended to functional similarity. It does however remain possible that if different fragments of FUS were tested in yeast they could demonstrate some prion-like behavior.

In most cases, as mentioned above, the self-replicating mechanism of prions is associated with a polymeric amyloid conformation, so if FUS or TDP-43 aggregates were propagating by such a mechanism, they would be expected to have characteristics of amyloid. However, neuronal protein inclusions in the case of ALS do not have properties typical of amyloids.⁸⁰ Consistent with this, cytoplasmic inclusions of TDP-43 and FUS in the yeast model have biophysical properties distinct from amyloid. Characterization of toxic TDP-43 aggregates in yeast indicated that they do not have the distinctive detergent resistance of amyloid, nor do they stain positive with the amyloid-reporting dye thioflavin-T.^{59,60} Similarly, FUS was found to form detergent-sensitive aggregates in yeast,^{58,61,62} and recombinant FUS formed aggregates that gave no positive thioflavin-T response.⁶³ However, Fushimi and coworkers found FUS aggregates in whole yeast cells to give a positive thioflavin-T response.⁵⁸

Intriguingly, SOD1 was recently shown to propagate like a prion through cell-to-cell transmission in Neuro-2a cells.⁸¹ The misfolding and aggregation of intracellular SOD1 was induced by an efficient internalization of preformed recombinant SOD1 aggregates. This implies an active seeding capability of internalized protein polymers; a feature that has been primarily attributed to amyloid aggregates. A similar seeding capability was reported for TDP-43 aggregates.⁸² While SOD1 and TDP-43 protein inclusions do not possess many of the defining characteristic features of amyloid,⁸⁰ they have been described as having a fiber-like morphology.⁸³⁻⁸⁵ Similar observations were reported for FUS inclusions.^{86,87} It is conceivable that pathological aggregates of TDP-43 and FUS might also share a common seeding mechanism of propagation with cell-to-cell transmission, which would explain the rapid progressive nature of ALS.⁸⁸

A pathogenic spreading mechanism was recently proposed for several major disease-linked protein aggregates, formed by A β , α -synuclein, Tau and polyglutamine.⁸⁹ Most of these proteins share no sequence similarity with yeast prion domains; for this reason the full-length FUS protein (as more aggregation-prone), rather than only its glutamine-rich domain, may be a better candidate for prion-like behavior. Because yeast is the most developed experimental system for studying prion propagation, it offers an obvious approach to testing if these disease proteins are capable of forming aggregates that can propagate like prions. It may be interesting to check whether native yeast Sod1 can acquire an aggregation state that is transmissible within a yeast population, which would make Sod1 the first described yeast prion without a glutamine/asparagine-rich prion domain. Likewise, propagation mechanisms of FUS and TDP-43 proteinopathies could be further examined in yeast, but ultimately such mechanisms will have to be determined in animal models.

High-Throughput Screening in Yeast Disease Models

Genetic screening. Perhaps the strongest attribute of yeast disease models is the potential for high-throughput screening. In the case of neurodegenerative disease models, the induced proteotoxicity provides an easy phenotypic readout to screen for genetic factors that can restore cell growth. Few model systems can equal the number of genetic tools available in yeast, including numerous genetic libraries and multiple yeast strain collections in which each of the open reading frames has been knocked out or modified. Coupled with fast and inexpensive culturing conditions, yeast can be used to quickly screen thousands of genetic modifiers, which can then be validated in higher disease models. For example, a yeast PD model identified the yeast protein Ypt1 as a suppressor of α -synuclein proteotoxicity.⁹⁰ The mammalian homolog of Ypt1, Rab1, is involved in vesicle trafficking and was confirmed to similarly suppress α -synuclein toxicity in flies, worms and neuronal cell culture. Similarly, the yeast ortholog of the PD-linked gene *ATP13A2* (also known as *PARK9*) was found as a potent high-copy suppressor of α -synuclein proteotoxicity;⁹¹ this protein also rescued dopaminergic neuron loss caused by α -synuclein overexpression in animal and neuronal PD models.⁹¹

Identification of specific proteins during yeast screens can also offer a complementary validation of factors that were previously suggested by independent methods, thus incidentally also identifying mechanisms that are conserved from yeast to mammals. For example, a genome-wide screen of loss-of-function mutations that suppress the toxicity of a mutant Huntingtin fragment identified Bna4, a conserved enzyme in the kynurenine pathway of tryptophan degradation, which had previously been linked to the pathology of Huntington disease.⁹²

In possibly the best illustration of the power of the yeast system, a link was established between familial ALS and mutations in the *ATXN2* gene.⁹³ A yeast TDP-43 proteinopathy model was used to screen a yeast genomic library for suppressors or enhancers of toxicity. One particular gene that was found to enhance TDP-43 proteotoxicity when overexpressed was *PBP1* (Pab1 Binding Protein 1), an ortholog of human Ataxin-2 (*ATXN2*). When the *PBP1* gene was deleted, there was a reduction in TDP-43 proteotoxicity, indicating that Pbp1 modifies TDP-43 toxicity in a dose-dependent manner.⁹³ These preliminary results in yeast were validated in a *Drosophila* fly model, and then in human cell culture it was demonstrated that TDP-43 and *ATXN2* physically interact via an RNA-dependent mechanism.⁹³ Subsequent analysis of spinal cord neurons of ALS patients revealed altered localization of *ATXN2* relative to normal controls. Genetic analysis of hundreds of ALS patients indicated intermediate polyglutamine expansion of *ATXN2* was significantly linked to a small percentage of ALS patients,⁹³ revealing an unknown genetic connection.

FUS proteinopathy yeast models have also been used for high-throughput genetic screening. Two recent approaches involved screening yeast open reading frame overexpression libraries, representing most of the approximately 6,000 yeast genes, for modifiers of FUS proteotoxicity.^{61,63} These separate screens recovered many of the same genes as capable of suppressing growth inhibition caused by FUS overexpression. Moreover, all of the common genes were DNA/RNA-binding proteins. Importantly, the identified set of genes was unique to FUS proteinopathy, distinct from what has been found to suppress other proteinopathies in yeast, indicating the specific mechanisms of toxicity associated with different disease proteins in yeast.

Genes identified as suppressing FUS proteotoxicity were further validated. One gene found to suppress FUS toxicity in both yeast screens was *ECM32*,^{61,63} the homolog of human *UPF1* that is implicated in many cellular processes involving RNA. When *UPF1* was expressed in yeast, it also ameliorated FUS proteotoxicity.⁶¹ Two additional yeast genes found by screening were *TIF2* and *CDC4*, the respective homologs of human *EIF4A1* and *FBXW7*.⁶³ Their specificity in FUS proteotoxicity suppression was confirmed when *EIF4A1* and *FBXW7* were similarly found to suppress FUS toxicity in human cell culture.⁶³

Chemical screening. In addition to screening for genetic modifiers, the yeast system is perfectly suited to perform large-scale chemical genomic screening. Robust and unfastidious growth of yeast cells offers a unique possibility to screen hundreds of thousands of small molecules in a single high-throughput experiment. Disadvantages include a limited permeability of some compounds due to special lipid composition of the yeast

plasma membrane, presence of a cell wall and pleiotropic drug transporters that can pump small molecules out of the cell. To circumvent these problems and maximize chemical library coverage, special yeast strains have been engineered using at least two different approaches. First, the deletion of the *ERG6* gene (sterol methyltransferase) was shown to inhibit ergosterol biosynthesis and enhances membrane fluidity, causing increased membrane permeability to various compounds.⁹⁴ Second, the deletion of two homologous transcription factors (*PDR1* and *PDR3* genes), which control the expression of pleiotropic drug resistance genes, disrupts active transport of small molecules outside the cell and leads to multiple drug sensitivity (reviewed in ref. 95).

Yeast models of neurodegenerative diseases have been successfully employed to identify small molecules capable of ameliorating or reversing toxicity in yeast associated with expression of α -synuclein^{33,96} and the polyglutamine-expanded fragment of the human Huntingtin protein.⁹⁷ Several potent compounds of different chemical nature have been identified, including natural polyphenol derivatives present in green tea⁹⁶ that were identified independently using different in vitro-based approaches.⁹⁸ Importantly, these compounds were successfully validated in fly and neuronal culture models, confirming the outstanding potential of the yeast system for initial inexpensive high-throughput screenings. Finally, the yeast models of TDP-43 and FUS proteinopathies offer near-absolute cell growth inhibition after overexpression (Fig. 2A), making them particularly suitable for high-throughput small molecule screenings.

Another approach was successfully developed by Marc Blondel's group, where native yeast amyloid-based prions were used as drug targets.⁹⁹ They have identified compounds that cause curing of yeast prions using a colorimetric (red/white)

reporter system, previously described for phenotypic detection of the [*PSI*⁺] prion.¹⁰⁰ Curiously, despite significant differences in structure between yeast and mammalian prions, many isolated drugs were active against mammalian prion in three different cell-based assays (reviewed in ref. 101), arguing that the mechanisms controlling prion propagation are conserved from yeast to humans. Two of the identified potent compounds, 6-aminophenanthridine and guanabenz,¹⁰² were also able to reduce aggregation of mutated Huntingtin and Poly(A) Binding Protein Nuclear 1 (PABPN1) in cell-based models of PD and oculopharyngeal muscular dystrophy, respectively.¹⁰³ These drugs were shown to bind to common specific positions in domain V of the large ribosomal RNA and affect the ability of the ribosome to assist protein folding.¹⁰⁴ Potentially, these anti-aggregation drugs may be beneficial for other protein folding diseases.

Concluding Remarks

Yeast models of human disease have obvious shortcomings and there will be questions about how closely the conditions in a yeast cell recapitulate conditions in differentiated human cells. Such doubts cannot be dismissed. However, the ease of use, the large number of tools and the overwhelming potential for very high-throughput genetic and chemical screens are powerful justification for using yeast models as a first-line approach to tackling exceptionally complicated human diseases. Specifically in the case of widespread neurodegenerative diseases, which exact a huge cost on society, the newly-developed yeast models provide a very inexpensive and fast approach to gather initial data that can be used to develop smart experimental strategies in higher organism models.

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