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Review Article

TDP-43 toxicity in yeast

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ABSTRACT

The budding yeast *Saccharomyces cerevisiae* is an emerging tool for investigating the molecular pathways that underpin several human neurodegenerative disorders associated with protein misfolding. Amyotrophic lateral sclerosis (ALS) is a devastating adult onset neurodegenerative disease primarily affecting motor neurons. The protein TDP-43 has recently been demonstrated to play an important role in the disease, however, the mechanisms by which TDP-43 contributes to pathogenesis are unclear. To explore the mechanistic details that result in aberrant accumulation of TDP-43 and to discover potential strategies for therapeutic intervention, we employed a yeast TDP-43 proteinopathy model system. These studies allowed us to determine the regions of TDP-43 required for aggregation and toxicity and to define the effects of ALS-linked mutant forms of TDP-43. We have also been able to harness the power of yeast genetics to identify potent modifiers of TDP-43 toxicity using high-throughput yeast genetic screens. Here, we describe the methods and approaches that we have used in order to gain insight into TDP-43 biology and its role in disease. These approaches are readily adaptable to other neurodegenerative disease proteins.

1. Introduction

1.1. Protein aggregation linked to neurodegenerative disease

As our population continues to age, neurodegenerative diseases will pose an increasing challenge to public health. Identifying and characterizing the specific disease proteins associated with these disorders will provide insight into disease pathogenesis and will aid the development of biomarkers and therapeutic strategies. Several devastating human neurodegenerative diseases are associated with the accumulation of protein aggregates in the brains of affected individuals. These include Alzheimer's disease (amyloid-beta and tau [1,2]), Parkinson's disease (α-synuclein [3]), prion diseases (PrP [4]), polyglutamine diseases (expanded polyglutamine [5]); and most recently ALS and frontotemporal lobar degeneration with ubiquitinated inclusions (FTLD-U) (TDP-43 [6]). Now that we know the identities of the aggregated disease proteins, the next step will be to understand at a mechanistic level how these proteins contribute to disease pathogenesis.

1.2. Yeast models of neurodegenerative disease

All cells have to deal with misfolded proteins, from simple yeast cells all the way to complex human neurons. Thus, it is likely that the mechanisms to cope with misfolded proteins as well as the cellular consequences of protein aggregation are conserved from yeast to man. Historically, yeast has been used to study many fundamental eukaryotic cellular pathways, including the cell cycle [7,8] and the secretory pathway [9–11], but also provides a tractable system to study pathways involved in dealing with misfolded and aggregated proteins [12], including those linked to human disease. Many yeast genes have human homologs and the core cellular pathways are well conserved, meaning that genetic interactions found in yeast are likely to be relevant to human disease [13]. The yeast genome is well characterized and easily manipulable (for example, deleting individual genes to probe their function). Thus, using a very simple but powerful genetic system we can define the pathways and genes that are affected by the excess accumulation of a neurodegenerative disease protein and develop innovative approaches to battle neurodegenerative disorders [12].

In the past few years, a number of yeast models of neurodegenerative diseases have been generated by overexpressing the wild type or mutant form of a human disease protein [14–16]. A yeast model of the Parkinson's disease protein α-synuclein has led to discoveries about its pathological properties [14]. The yeast α-synuclein model has also provided insight into conserved vesicle trafficking pathways affected by α-synuclein accumulation

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[17,18] as well as to suggest novel connections between genetic and environmental contributors to Parkinson's disease [19]. Importantly, results from the yeast studies have been validated in animal and cellular models [17–21]. Notably, the relevance of an overexpression model of even the wild type form of the protein is highlighted by (1) the observation that wild type protein accumulates in most cases of disease and the mutant forms are much rarer and (2) the discovery of duplication and triplications of the α-synuclein gene in rare familial forms of Parkinson's disease [22–24].

Yeast models have also been created to model polyglutamine (polyQ) disorders (e.g. Huntington's disease), including expression of a fragment of the huntingtin protein with and without polyQ expansions [15,25]. These studies have recapitulated the polyQ-length dependent aggregation and toxicity as well as to begin to uncover key early pathways affected by polyQ aggregation [26]. Because TDP-43 has emerged as a key player in ALS and FTLD-U (see later), we have recently generated a new yeast model to study TDP-43 [16,27] and describe various experimental approaches used to study critical features of TDP-43 aggregation and toxicity as well as techniques used to identify modifier genes.

1.3. TDP-43 is the major disease protein in ALS and FTLD-U

In 2006, Lee and colleagues discovered that the TAR DNA binding protein (TDP-43) was the abnormally accumulated protein in amyotrophic lateral sclerosis (ALS), which is also known as Lou Gehrig's disease, and frontotemporal lobar degeneration with ubiquitinated inclusions (FTLD-U) [6]. Subsequent work by a number of groups identified mutations in the TDP-43 gene (*TARDBP*) in some familial and sporadic cases [28–32]. Thus, human genetics and pathology have both converged on TDP-43 as playing a critical role in disease pathogenesis [33]. However, very little is known about the cellular pathways affected by TDP-43 in disease. Understanding these critical pathways will be essential for developing effective therapeutic interventions. TDP-43 is a nuclear RNA binding protein that shuttles between the nucleus and the cytoplasm [34,35]. In affected neurons, TDP-43 accumulates in the cytoplasm and is depleted from the nucleus, suggesting that a change in subcellular localization might be important for disease pathogenesis. Despite major efforts to understand the biology of TDP-43 we currently do not know how loss of the normal functions of TDP-43 or perhaps a gain of toxic function might contribute to disease. To address these deficits, we have been exploring TDP-43 aggregation and toxicity using the yeast model system.

1.4. Yeast TDP-43 proteinopathy model

We first sought to determine if we could recapitulate key aspects of TDP-43 biology in yeast cells, including cytoplasmic aggregation and toxicity. To regulate the expression of TDP-43 we placed a single copy of the human gene into a yeast strain under the control of a tightly regulated galactose-inducible promoter. That allowed us to maintain the cells in glucose, to repress TDP-43 expression, and then to rapidly and synchronously induce expression in all cells of the culture. To allow *in vivo* visualization of the protein in real time, and therefore monitor its propensity to form aggregates, we generated constructs that contain TDP-43 fused to the green fluorescent protein (GFP). To generate these constructs, a TDP-43 Gateway® entry clone was obtained from Invitrogen that contained the full-length human TDP-43 cDNA in the pDONR221 vector. We used this TDP-43 entry clone in Gateway LR reactions to shuttle TDP-43 into Gateway-compatible yeast expression vectors (http://www.addgene.org/yeast_gateway; [36]). To tag TDP-43 with GFP we created new entry clones lacking stop codons (TDP-43_{nostop}) and used these in LR reactions with pAG426Gal-ccdB-GFP to generate the 2-micron (2μ) TDP-43-GFP fusion constructs. To generate the integrating TDP-43-GFP construct the TDP-43_{nostop} entry clone was used in an LR reaction with pAG306Gal-ccdB-GFP. 2μ plasmid constructs (e.g. pAG426Gal-TDP-43-GFP) were subsequently transformed into BY4741 yeast strains (*MATa his3 leu2 met15 ura3*) and the integrating TDP-43-GFP constructs were transformed into the W303 strain (*MATa can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 ade2-1*). For yeast transformation we used the PEG/Lithium acetate method according to standard protocols [37].

Having generated a panel of TDP-43 yeast expression constructs, we next investigated the effect of TDP-43 expression in living cells. To assess TDP-43 toxicity, we performed spotting assays. For spotting assays, 3 ml of yeast culture were grown overnight at 30 °C in liquid media containing raffinose (SRaf/-Ura) until they reached log or mid-log phase. Growth in raffinose does not activate the galactose-inducible promoter but also does not repress it, thus priming the yeast cells for rapid induction upon addition of galactose. Cultures were subsequently normalized for cell number, serially diluted and spotted onto synthetic solid media containing either glucose or galactose lacking uracil and were grown at 30 °C for 2–3 days. Expression of TDP-43-GFP from a 2-μ plasmid led to inhibition of cell growth compared to cells transformed with a plasmid containing GFP alone (Fig. 1A). Thus, TDP-43 expression in yeast resulted in cellular toxicity.

We next considered if TDP-43 would form cytoplasmic aggregates in yeast, similar to those observed in human disease and performed experiments to visualize TDP-43-GFP localization. For fluorescence microscopy experiments, we began with a yeast strain harboring an integrated TDP-43-GFP construct. Single colony isolates of the yeast strains were grown to mid-log phase in SRaf/-Ura media at 30 °C. Cultures were spun down and resuspended in SGal/-Ura to induce expression of the TDP-43 constructs. Cultures were induced with galactose for 4–6 h, fixed using ethanol and counter-stained with DAPI, to visualize nuclei. At early time points (3–4 h) after induction, GFP alone was diffusely distributed between the cytoplasm and the nucleus, whereas the

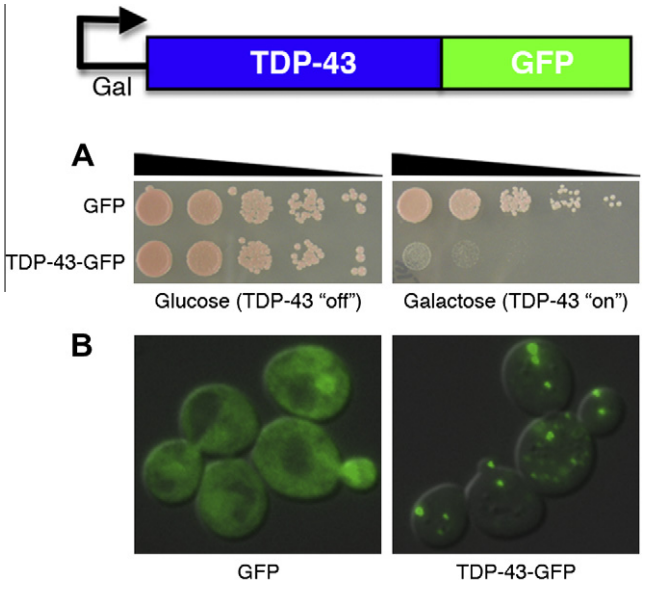

Fig. 1. Yeast TDP-43 proteinopathy model. A TDP-43 expression construct under the control of a tightly regulated galactose-inducible promoter was introduced into yeast cells. (A) Spotting assays using five-fold serial dilutions demonstrate TDP-43 is toxic when expressed at high levels in yeast cells (pAG426Gal-TDP-43-GFP). When cells are spotted on plates containing glucose, TDP-43 expression is repressed and induced in the presence of galactose. (B) TDP-43-GFP formed multiple cytoplasmic foci when expressed in yeast cells, whereas GFP alone was diffusely distributed throughout the cytoplasm and nucleus.