UNIVERSITY OF CALIFORNIA SAN DIEGO

Controlled TDP43 Expression in *S. Cerevisiae* Exhibits Age Dependent Toxicity

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by

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Professor Randolph Hampton, Co-Chair
Professor Lisa McDonnell

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The thesis of Taylor Wall is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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University of California San Diego

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ABSTRACT OF THE THESIS

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As cells age they develop distinctive characteristics that are highly associated with diseases like amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). One of those highly associated characteristics is the formation of toxic protein aggregates like transactive response DNA binding protein (TDP43) that lead to disease pathology. To study the effects of TDP43 expression in aging cells we used growth assays, which showed that increased

TDP43 expression is toxic to yeast cells. Our microscopy of aging cells expressing TDP43 also revealed that as cells age TDP43 expression becomes toxic, and nuclear expression seems to decrease as TDP43 expression moves to the cytoplasm and forms pathological aggregates. Due to the many important roles of TDP43 in the nucleus in its stable form, we hypothesized that in young cells low TDP43 expression wouldn't be toxic but would become toxic as the cells aged. We found that at low expression levels of TDP43 we were able to maximize the amount of time at which we could image the aging cells before they died from the toxicity. This poses a possible mechanism by which we can observe important TDP43 expression characteristics for the entirety of the cell's lifespan.

I. INTRODUCTION

A. *Saccharomyces cerevisiae* as a model for aging

Saccharomyces cerevisiae (yeast) is a widely used model for aging studies due to its relatively short life span in comparison to immortalized human cell lines and murine model systems. Yeast can be more readily used for cellular aging studies than murine model systems, and their entire life span can be followed in an experimentally friendly amount of time (2-3 days). There is also evidence that many age-preventative mechanisms like caloric deficit and the inhibition of mammalian target of rapamycin (mTOR) signaling are conserved in yeast as well as mammals [12]. Aging in yeast culminates in the formation of extrachromosomal ribosomal deoxyribonucleic acid (DNA) circles (ERCs), protein aggregates, and oxidatively damaged proteins [1]. These formations lead to cellular toxicity and senescence of the mother cells as more and more of them accumulate in the cell. Age is a risk that directly correlates with neurodegenerative diseases like amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Yeast can be used to model some of these hallmark disease characteristics, like the formation of transactive response DNA binding protein (TDP43) aggregates in ALS and FTD as people age [3]. Using a mother enrichment program (Mep), the replicative lifespan (RLS) of yeast mother cells can be followed as they age without the need to use micromanipulation, which is tedious and requires more time. It utilizes a Cre-*lox* recombination system that disrupts two essential genes that are important in cell cycle progression in the daughter cells so that they remain in M-phase (Fig. 1) [2]. This allows for the mother cells to be studied under a microscope in real time as they age, so that the effects of TDP43 on aging cells can be observed. TDP43 inhibition by eIF2a-phosphorylation has also been shown to reduce toxicity in *D. Melanogaster* as well as mammalian cells, providing more evidence for its relevance in other studies [11].

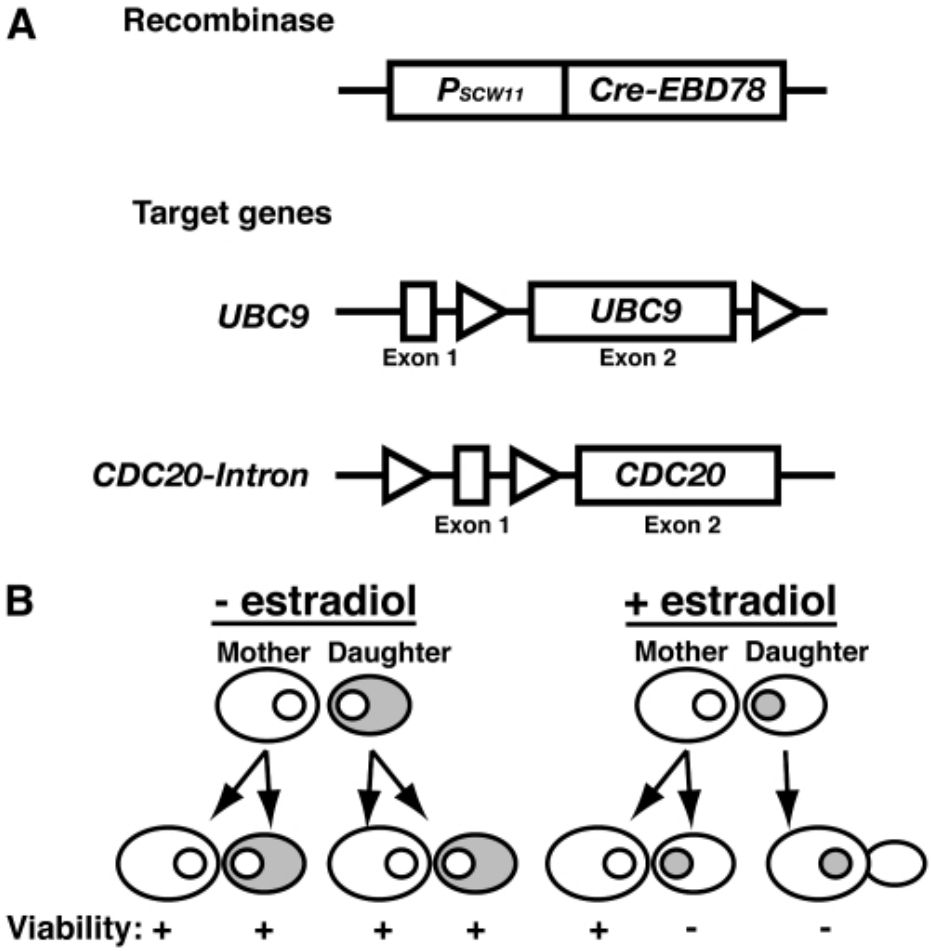


Figure 1. Components of the MEP. (A) Diagram of the MEP components. Cre-EBD78 is a novel version of a fusion protein between the Cre recombinase from bacteriophage P1 and the estrogen-binding domain of the murine estrogen receptor that is strictly dependent on estradiol for activity (see materials and methods). PSCW11-cre-EBD78 is integrated at the ho locus and specifically expressed in newborn cells, using a daughter-specific promoter derived from SCW11. Target genes were constructed at their endogenous loci by introduction of loxP sites (triangles). For UBC9, recombination between the loxP sites removes exon 2, representing 92% of the coding region. For CDC20, an intron derived from ACT1 and containing a loxP site was introduced to generate a 42-bp exon 1. Recombination between loxP sites removes exon 1, leaving the first in-frame start codon at methionine 197, which would generate a nonfunctional protein. (B) Illustration of the expected localization of Cre-EBD78 (shading) in response to estradiol. PSCW11-cre-EBD78 expression is restricted to the G1 phase of daughter cells. In the absence of estradiol the fusion protein is sequestered in the cytoplasm. Upon ligand binding, the fusion protein is translocated into the nucleus (small circle), where it can act on loxP target sites. (Lindstrom et. al. 2009)

B. TDP43 leads to cellular toxicity in age-related neurodegenerative diseases

TDP43 is a transactive DNA binding protein that consists of an N-terminal domain with a nuclear localization signal (NLS), two ribonucleic acid (RNA) recognition motifs (RRMs), and a conserved, glycine rich C-terminal region [3]. The C-terminal region is the site of many mutations that are associated with the accumulation of TDP43 protein aggregates in ALS and FTD, which usually form in the neurons and glia in humans. It also contains a low complexity domain (LCD) that suggests prion like behavior and is thought to be a driving force behind the mislocalization and aggregation of TDP43 [8]. TDP43 pathology is linked to the mislocalization of this protein from the nucleus to the cytoplasm, which leads to the formation of stress granules and usually precedes some level of cellular toxicity (Fig. 2) [3]. It is not known if the cellular mislocalization of TDP43 is a causal factor in ALS or FTD pathology, but many mutations in the TDP43 LCD are directly related to those diseases and the formation of these aggregates [4]. Once in the cytoplasm it has been observed to form stress granules composed of protein and RNA. The function of stress granules has yet to be discovered, but they are thought to function in protecting RNA when the cell is under stress. Evidence suggests that the C-terminal domain and the two RRMs are both needed for the mislocalization and aggregate formation in the cytoplasm, which teases a possible region of TDP43 that could be involved in cellular toxicity [3].

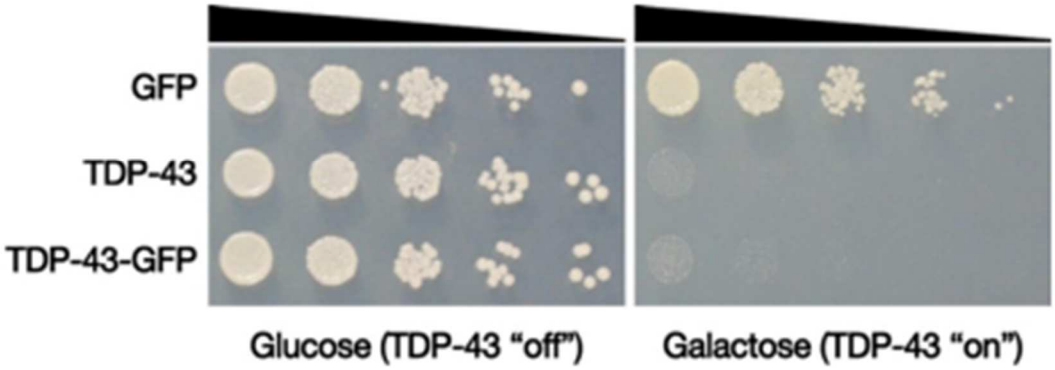


Figure 2. TDP-43 is toxic to yeast cells. Yeast cells were transformed with a galactose-inducible GFP construct, untagged TDP-43, or a TDP-43-GFP fusion. Serial dilutions of transformants were spotted on glucose- or galactose containing agar plates, and growth was assessed after 2 days. Whereas the transformants grew equally well on the control glucose plates, TDP-43 expression profoundly affected growth. (Johnson et. al. 2008)

C. TDP43 pathology in ALS and FTD

ALS and the most common subtype of FTD (FTLD-U) are characterized by the formation of pathological TDP43 inclusions. It was discovered that C-terminal fragments of TDP43 that are hyperphosphorylated and ubiquitinated form these cellular inclusions in glia and neurons [5]. TDP43 in its stable form in the nucleus functions to regulate transcription, stabilize mRNA, and regulate splicing [3]. As previously stated, the mislocalization of TDP43 to the cytoplasm is a defining feature of these inclusions in ALS and FTD. It was discovered as the "pathological protein" in these diseases using post-mortem brain tissue to obtain peptides that corresponded to the C-terminal region of TDP43 (Fig. 3) [5]. Antibodies for TDP43 were also used to tag the inclusions in ALS and FTD. Potential phosphorylation sites were also discovered in the highly conserved C-terminal region of TDP43 that may function to help drive aggregation. Although it has been determined to be pathogenic in these neurodegenerative diseases, the specific role it plays as a loss of function or gain of function protein is still being studied [5].

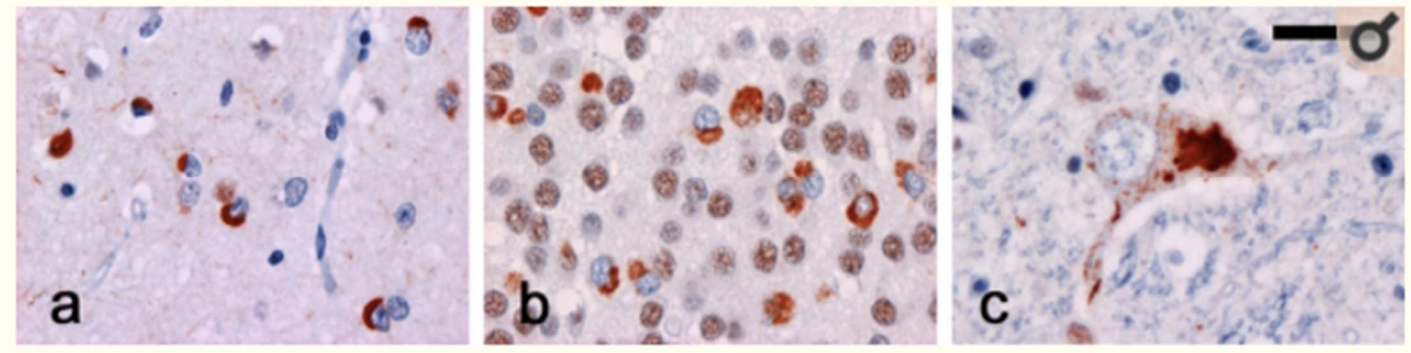


Figure 3. TDP-43 immunohistochemistry in frontotemporal lobar degeneration with ubiquitinated inclusions (FTLD-U) and amyotrophic lateral sclerosis (ALS)
A polyclonal antibody against TDP-43 labels neuronal inclusions in (a) neocortex and (b) hippocampus in FTLD-U and (c) lower motor neurons in ALS. Note that cells with inclusions show loss of normal diffuse nuclear staining. TDP-43 immunohistochemistry. Scale bar; (a,b) 20 μm, (c) 15μm. (Mackenzie et. al. 2008)

D. Advantages of using tetracycline-controlled transcriptional activation

In previous studies performed observing the effects of TDP43 expression on yeast cells, a galactose (GAL) inducible promoter was used to drive expression [3]. While this design was able to determine that overexpression of TDP43 in yeast resulted in cellular toxicity and cytoplasmic mislocalization, it didn't provide for a specific and controlled way to study the effects of varying expression levels of TDP43. To control for this a tetracycline-controlled transcriptional activator (rtta) was used that is able to bind to doxycycline. When the rtta is bound to doxycycline, only then is it able to bind the tetracycline response element (TRE) which consists of an operator and a promoter that are placed upstream of the gene that is being expressed [6]. In this Tet-ON system, the expression of the gene of interest is being increased.

E. Focus of project

TDP43 has been proven to be a significant and relevant protein to study when looking at age related neurodegenerative diseases like ALS and FTD. Using a doxycycline-controlled Tet-ON system, the expression of TDP43 can be increased in a dose dependent manner and provide a much more specific approach to observing its role in cellular toxicity. This coupled with a Mep strain that allows us to look at yeast cells in real time as they age can provide further clarity about the pathological role TDP43 plays.

We expect that since TDP43 in its stable form in the nucleus has important cellular functions, that means younger cells that express TDP43 will not display those characteristics of pathology seen in ALS and FTD. Age is a risk that is directly related to these diseases, which is why we want to study a model that allows us to look at that. We predict that if we express TDP43 in young cells and watch them age, the older cells will display increased TDP43 aggregation in the cytoplasm. We also predict that we will see a lot of TDP43 expression in the nucleus since that is where it is normally localized.

Based on the toxicity observed in the galactose inducible TDP43 overexpression experiment performed, we hypothesize that there is a "sweet spot" we can hit where the concentration of TDP43 is low enough that in young cells it doesn't cause toxicity, and in old cells it starts to exhibit signs of pathology without killing the cells outright (Fig. 2) [3].

II. MATERIALS AND METHODS

A. Constructs used to test TDP43 expression in aging cells

1. The first construct used was the mep strain in yeast background BY4761 to control for the effects of TDP43 expression (Fig. 4A).

2. The second construct used was a mep strain (background BY4761) that was transformed with rtta-TDP43 using the LiAc/SS-DNA/PEG method [7]. This construct was used to observe TDP43 expression in aging yeast cells (Fig. 4B).

A) 

B) 

Figure 4. Figure showing TDP43 constructs A) Mep background strain used to control for TDP43 B) Mep+rtta+TDP43 experimental strain used to look at TDP43 expression in aging mother cells

B. Growth assay performed in liquid culture

1. The mep+rtta+TDP43 strain and the mep strain were inoculated in synthetic medium plus glucose (SC+) and allowed to grow overnight at 30^{o}C. In the morning, the OD_{600} was measured using a Thermo Scientific Spectronic 200 Spectrophotometer. The samples were diluted back and allowed to double at least two to three times before reaching an OD_{600} of between 0.2 and 0.5.

2. Once they reached the desired OD_{600}, estradiol (1000x) was added to one experiment but not the other. In each experiment doxycycline was added at concentrations of 0, 2, 10, or 50 ug/mL. The cultures were grown at 30^{o}C, and the OD_{600} was measured every couple of hours to determine the growth of the cells.

C. Mother enrichment protocol

1. In order to label cells with Dylight633, the Dylight633 is diluted 1000x in 1mL of PBS+glucose 2% for each sample. Cells are spun down for 1 minute at 3000rpm to wash out the SC+ media, then they are washed twice with 300uL of pre-warmed PBS+glucose 2%. The cells are then resuspended in 1mL of PBS+glucose 2%+Dylight633 and incubated at 30ºC for 15 minutes.

2. The cells are then resuspended in SC+ media and diluted 1000x and allowed to recover at 30ºC for 1 hour. Estradiol (1000x) is then added to initiate the mep protocol, as well as varying concentrations of doxycycline depending on the experiment.

D. Growth plate assays

1. The mep+rtta+TDP43 strain and the mep strain were inoculated in synthetic medium plus glucose (SC+) and allowed to grow overnight at 30^{o}C. In the morning, the OD_{600} was measured using a Thermo Scientific Spectronic 200 Spectrophotometer. The samples were diluted back and allowed to double at least two to three times before reaching an OD_{600} of between 0.2 and 0.5.

2. Once they reached the desired OD_{600}, estradiol (1000x) was added to one experiment but not the other. In each experiment doxycycline was added at concentrations of 0, 2, 5, 8, 10, or 50 ug/mL.

3. The experiment with estradiol grew for 24 hours in liquid culture before samples were taken and the media was washed out. The samples were resuspended in water and diluted 10000x before being plated on YPD plates.

4. The experiment without estradiol was diluted 10000x and plated on YPD plates.

5. Both experiments were grown on YPD plates at 30^{o}C for 2-3 days after which the number of colonies were counted.

E. Microscopy of mep+rtta+TDP43 strain

1. The mep+rtta+TDP43 strain was inoculated overnight in SC+glucose medium at 30ºC and diluted back in the morning. Following two to three doublings, once the OD_{600} reached 0.2-0.5 the mep protocol was initiated as previously stated.

2. The mep+rtta+TDP43 strain was continuously grown for 3 days at 30ºC, and samples were taken out to image at various time points. The cells were imaged using an Echo Revolve microscope at 60x objective with Cy5, mcherry, and brightfield channels (Fig. 5).

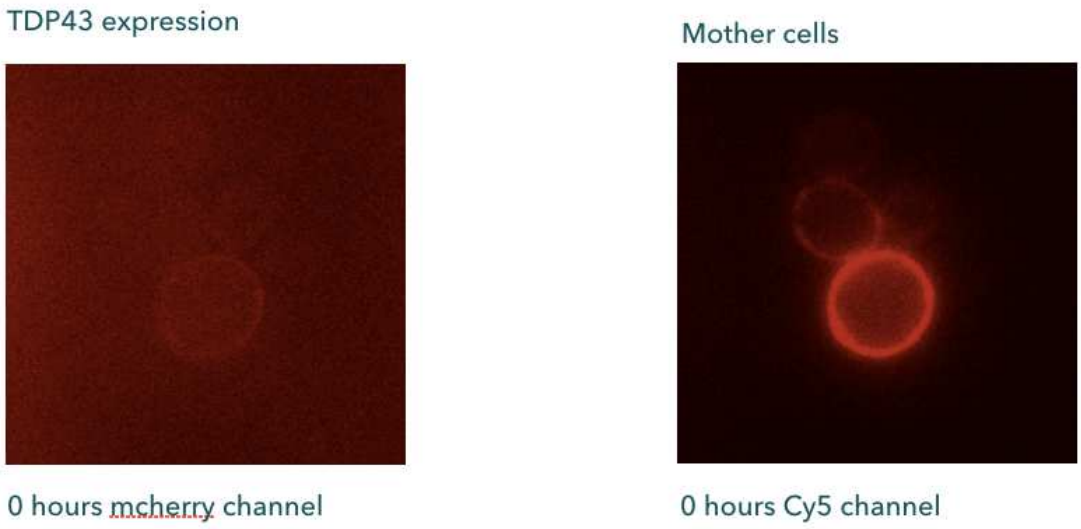


Figure 5. Microscopy images of a mother cell wall dyed with Dylight633 in the Cy5 channel, and the corresponding image showing TDP43 expression in the mcherry channel at 0 hours and 0 ug/mL of dox

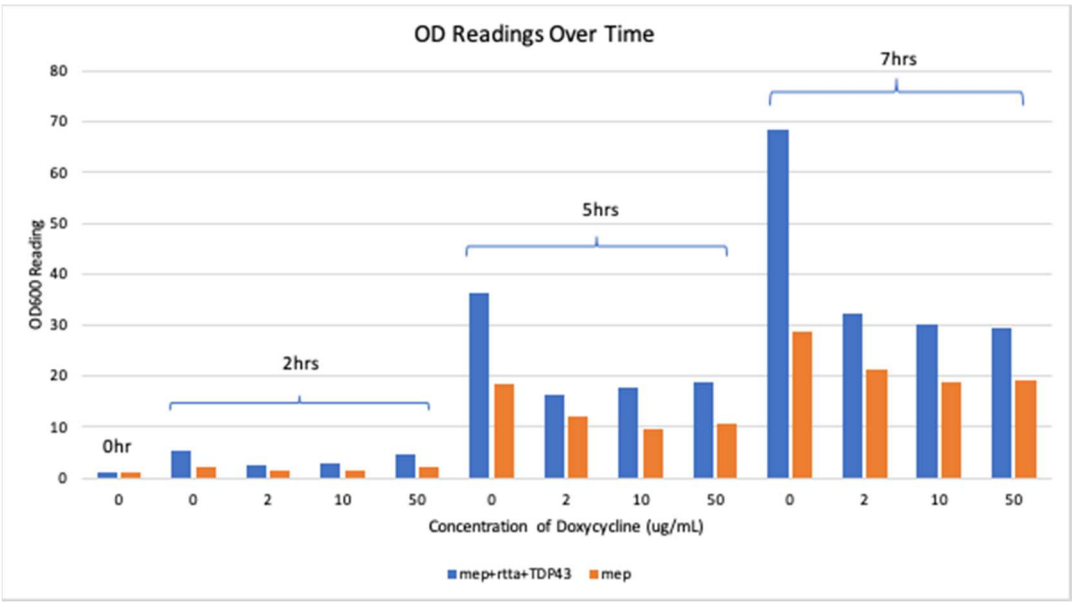
III. RESULTS

A. Quantification of TDP43 expression in liquid culture

The mep+rtta+TDP43 strain and mep strain were inoculated in synthetic complete medium plus glucose and allowed to grow overnight at thirty degrees Celsius. In the morning they were diluted back and allowed to grow up to an OD_{600} of around 0.2-0.5. Once they reached OD_{600} around 0.2-0.5, doxycycline was added at concentrations of 0, 2, 10, and 50 ug/mL. Estradiol (1000x) was also added and their OD_{600} was measured every couple of hours to observe the growth. In each graph shown the readings were normalized to the time zero OD_{600} (Fig. 6).

Both data sets followed the same trend, with the mep+rtta+TDP43 strain growing at a faster rate than the mep strain. The concentrations of doxycycline (dox) used increased from 0 ug/mL to 50 ug/mL. As the concentration of doxycycline increased, the OD_{600} readings for both the mep+rtta+TDP43 strain and the mep strain decreased. For the mep+rtta+TDP43 strain, there was an almost two-fold decrease between the readings at 0 ug/mL and 50 ug/mL (Fig. 6). This was expected because TDP43 expression has been shown to be toxic to yeast cells, and to impair cellular growth. In the mep strain there was a less dramatic stepwise decrease in cellular growth, which was expected as it is not expressing TDP43. The decrease in cellular growth of the mep strain can be attributed to the general toxicity of estradiol when added to yeast cells.

A).



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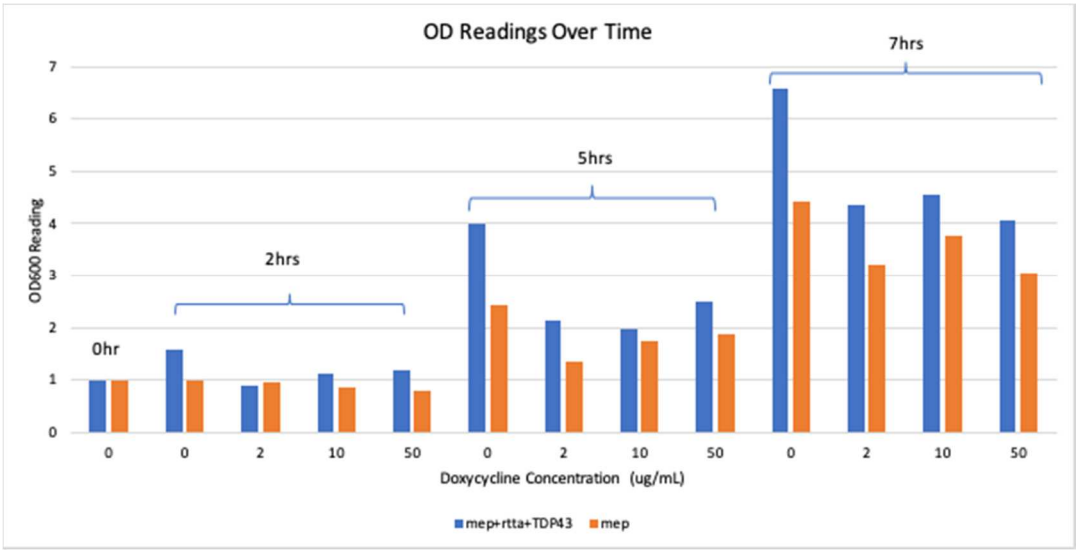


Figure 6. OD_{600} readings normalized to time zero A) Quantification of the OD_{600} readings of the mep+rtta+TDP43 strain and mep strain from 0 to 7 hours and 0 to 50 ug/mL of dox B) Graph B shows the same values but on a smaller scale due to normalization

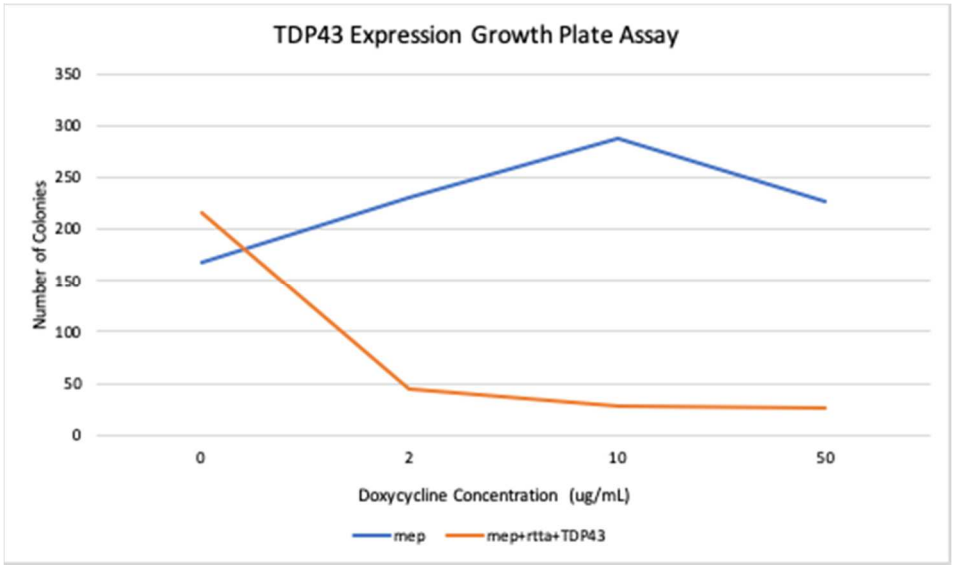
B. Quantification of TDP43 expression using growth plate assays

The mep+rtta+TDP43 strain and mep strain were inoculated overnight in a liquid culture of synthetic complete medium plus glucose and diluted back in the morning. The cultures were allowed to grow to an OD_{600} of around 0.2-0.5, and then doxycycline was added in concentrations of 0, 2, 5, 8, 10, and 50 ug/mL of dox. Estradiol (1000x) was added in one experiment, but not in the other experiment. The experiment that was grown with estradiol for 24 hours contained 0, 2, 5, and 8 ug/mL of doxycycline (Fig. 7B). The experiment without estradiol contained 0, 2, 10, and 50 ug/mL of doxycycline (Fig. 7A).

In the plates with no estradiol, the mep+rtta+TDP43 strain showed a sharp decline in the number of colonies from 0 to 2 ug/mL of doxycycline, and once it reached 10 ug/mL the number of colonies continued to decrease much more gradually and flatten out (Fig. 7A). This is expected because the samples with higher concentrations of doxycycline were expressing more TDP43 than the lower concentrations, and this results in an increased level of cellular toxicity. The mep strain showed a steady increase in colony numbers until it reached 10 ug/mL, where the number of colonies started to decline. This is unexpected because without the addition of estradiol or the presence of TDP43, the number of colonies should be around the same amount regardless of whether or not doxycycline was added (Fig. 7A).

The mep+rtta+TDP43 strain was grown in estradiol + doxycycline media for 24 hours before the media was washed out and the cells were plated. It shows a big decrease in colony numbers from 0 hours to 24 hours, and zero colony growth at concentrations larger than 2 ug/mL (Fig. 7B). This is expected because TDP43 causes cellular toxicity at higher expression levels as cells age. The reduction in colony growth at 0 ug/mL at 24 hours can be attributed to the general toxicity of estradiol.

A).



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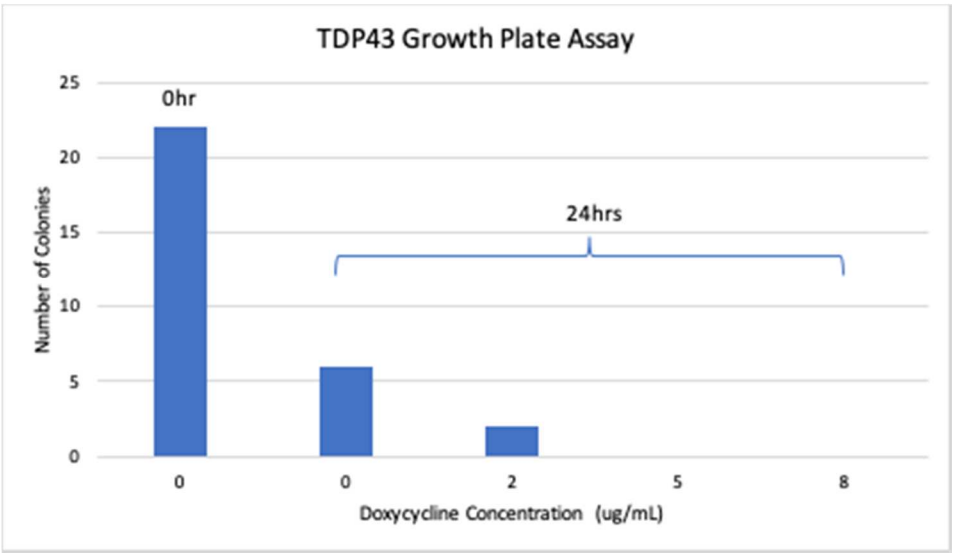


Figure 7. TDP43 expression shown using colony growth A) Quantification of colony growth of the mep+rtta+TDP43 strain and mep strain at 0, 2, 10, and 50 ug/mL of dox B) Quantification of colony growth of the mep+rtta+TDP43 strain after 24 hours at 0, 2, 5, and 8 ug/mL of dox

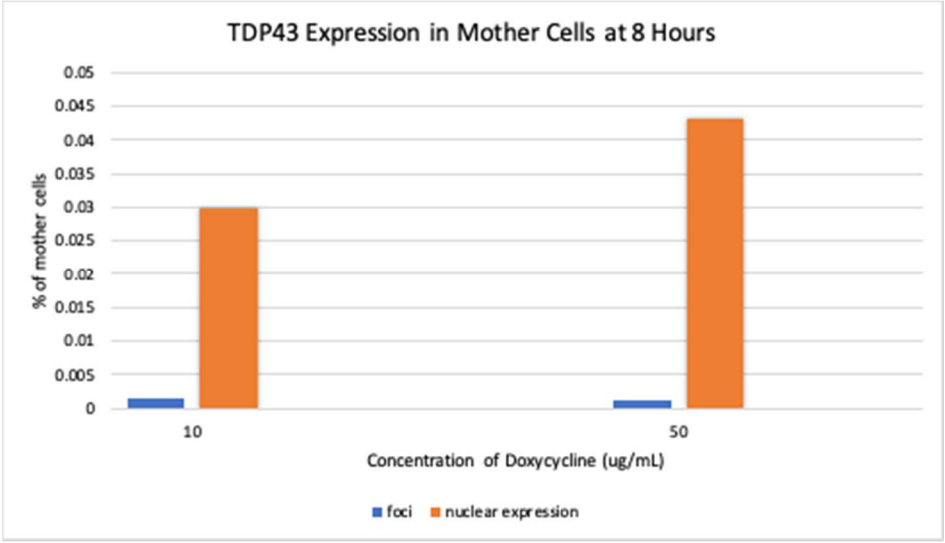
C. Microscopy to quantify TDP43 expression and toxicity

Cells were inoculated and grown up to an OD_{600} of 0.2-0.5 as described in previous experiments. Using the mep+rtta+TDP43 strain, the Mep protocol was initiated and cells were imaged at varying times over the course of 2-3 days. Doxycycline was added in concentrations of 0, 2, 10, and 50 ug/mL to the cells to observe the effects of TDP43 expression. The images taken were used to quantify the number of mother cells with foci and the number of mother cells with TDP43 expression in the nucleus. This was normalized by dividing the sum of the total number of mother cells with foci or nuclear TDP43 expression by the total number of cells observed.

TDP43 expression in mother cells at 8 hours (Fig. 8A) at 10 and 50 ug/mL of dox showed the same percent of mother cells with foci formation, and a slightly larger percent of mother cells with nuclear TDP43 expression. This provides evidence that younger cells don't have as much toxic protein aggregation caused by TDP43 expression regardless of how much dox was added. There was a larger percent of mother cells with nuclear TDP43 expression at 50 ug/mL of dox, which was expected because TDP43 is normally expressed in the nucleus in younger cells. The increased amount of dox can account for the difference in the percent of mother cells with nuclear expression because those cells were expressing more TDP43 (Fig. 8A).

TDP43 expression in mother cells at 24 and 44 hours at 2 ug/mL of dox showed a significant increase in the percent of mother cells with foci formation (Fig. 8B) compared to at 8 hours. There was also a significant decrease in the percent of cells with nuclear expression from 24 to 44 hours. The increase in the percent of cells with foci formation is expected as the cells age and TDP43 expression localizes to the cytoplasm and becomes toxic. The decrease in the percent of cells with nuclear expression from 24 to 44 hours is also expected as TDP43 mislocalizes to the cytoplasm as it becomes toxic and forms foci (Fig. 8B).

A).



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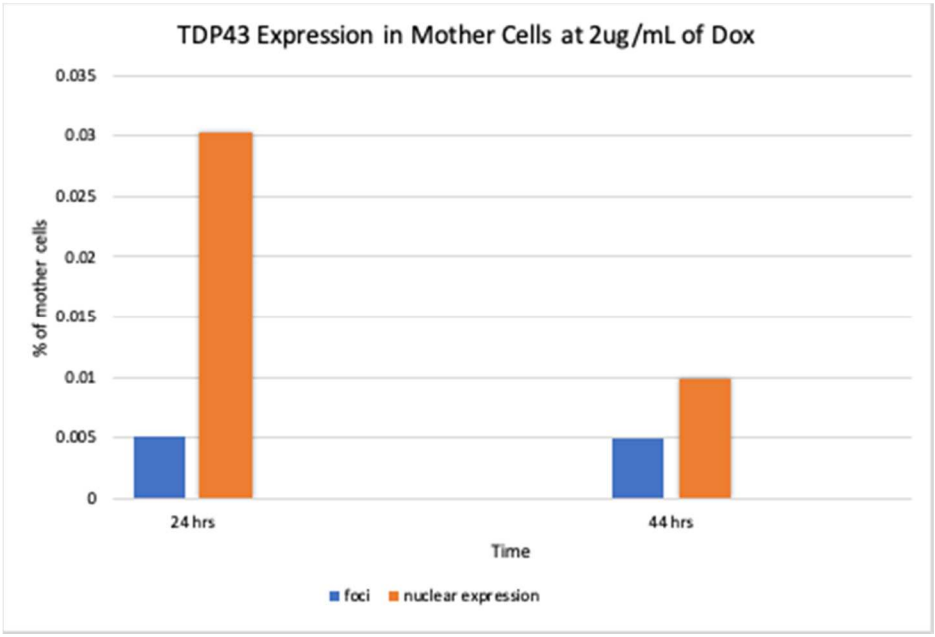


Figure 8. Percent of mother cells with TDP43 expression A) Percent of mother cells with foci and nuclear expression at 8 hours at 10 and 50 ug/mL of dox B) Percent of mother cells with foci and with nuclear expression at 24 and 44 hours at 2 ug/mL of dox

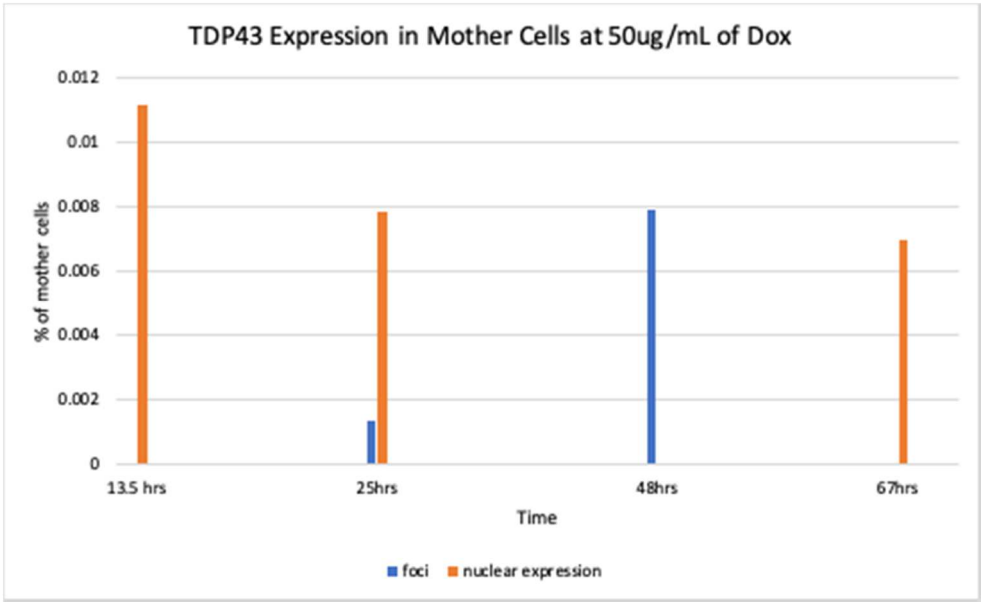
D. Microscopy to quantify TDP43 expression and toxicity

Cells were inoculated and grown up to an OD_{600} of 0.2-0.5 as described in previous experiments. Using the mep+rtta+TDP43 strain, the Mep protocol was initiated and cells were imaged at varying times over the course of 2-3 days. Doxycycline was added in concentrations of 10 and 50 ug/mL to the cells to observe the effects of TDP43 expression. The images taken were used to quantify the number of mother cells with foci and the number of mother cells with TDP43 expression in the nucleus. This was normalized by dividing the sum of the total number of mother cells with foci or nuclear TDP43 expression by the total number of cells observed.

At 50 ug/mL of dox there is a significant increase in the percent of mother cells with foci from 13.5 to 48 hours (Fig. 9A). There is also a gradual decrease in the percent of mother cells with nuclear expression from 13.5 to 67 hours. The increase in the percent of mothers with foci formation is expected as the cells age and TDP43 expression becomes more toxic, especially at higher expression levels like 50 ug/mL. The decrease in the percent of mothers with nuclear expression is also expected as cells age and TDP43 starts to aggregate and localize in the cytoplasm (Fig. 9A).

At 10 ug/mL of dox there is a similar percent of mothers with foci at 13.5 and 67 hours (Fig. 9B). There is also a significant decrease in the percent of mothers with nuclear TDP43 expression from 13.5 to 48 hours. An increase in the percent of mothers with foci is expected, but here a similar percent of mothers with foci is seen. The decrease in percent of mothers with nuclear expression is expected as TDP43 mislocalizes to the cytoplasm (Fig. 9B). Interestingly, there seems to be a significant percent of mother cells with foci present at 67 hours at 10 ug/mL, but not at 50 ug/mL. This could be due to the higher TDP43 expression killing off the cells at 50 ug/mL of dox before the foci are visible.

A)



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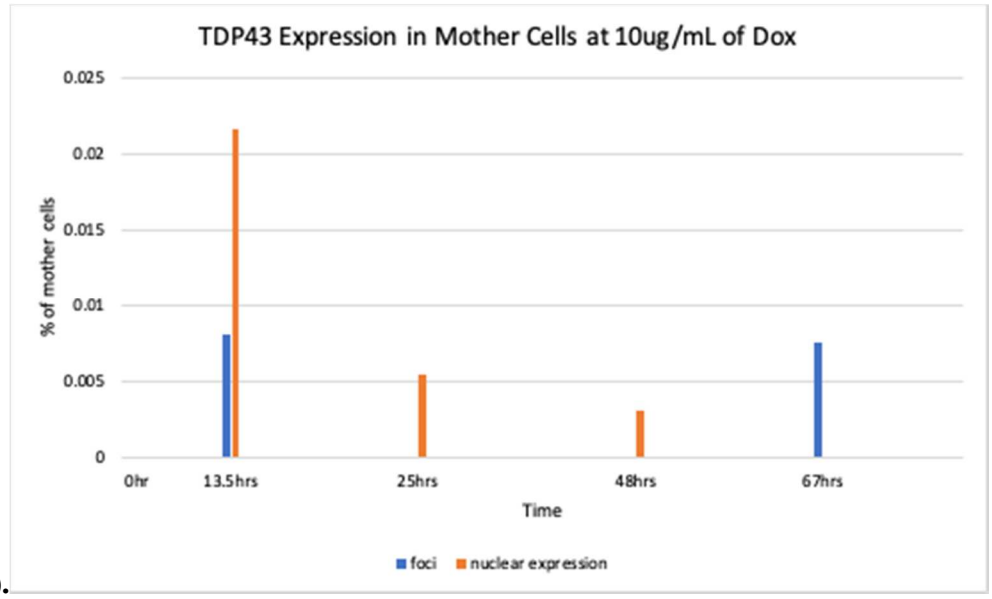

Figure 9. Percent of mother cells with TDP43 expression A) Percent of mother cells with foci and nuclear TDP43 expression at 13.5, 25, 48, and 67 hours at 50 ug/mL of dox B) Percent of mother cells with foci and nuclear expression at 13.5, 25, 48, and 67 hours at 10 ug/mL of dox

IV. Discussion

A. TDP43 expression becomes toxic to yeast as they age

Looking at the data from the liquid growth assays (Fig. 6), we can see that as TDP43 expression increases the OD_{600} of the mep+rtta+TDP43 strain decreases. We can also see that as the concentration of dox increases the OD_{600} of the mep strain shows a slight decrease but keeps a more constant level. This is due to the fact that the mep strain isn't expressing TDP43, so the lack of expression shouldn't cause the toxicity that we see in the mep+rtta+TDP43 strain. In our growth plate assays we also see something similar (Fig. 7A) where in our mep+rtta+TDP43 strain there is a sharp decline in colony growth from 0 to 2 ug/mL of dox despite the low expression of TDP43. This indicates that even in low levels TDP43 is toxic to cells but seems to level out after 2 ug/mL hinting that this could be a possible candidate for the optimum TDP43 expression. We also see this in our growth plate assay where the cells were grown in liquid medium with estradiol and dox for 24 hours (Fig. 7B). In this experiment we can see that past 2 ug/mL there is zero colony growth at 5 and 8 ug/mL, also lending to the hypothesis that 2 ug/mL provides the optimum expression of TDP43. More data needs to be collected to confirm the optimal concentration of dox.

B. TDP43 expression hints at a specific phenotype as cells age

In our microscopy data we can see that the percent of mother cells with nuclear expression is higher in younger cells but begins to decline as the cells age (Fig. 8B). This is expected because TDP43 performs vital functions in the nucleus of the cell in its stable form but becomes toxic as the cell ages. This decline in the percent of mother cells with nuclear expression is also seen over time at 10 and 50 ug/mL (Fig. 9), showing that the trend exists whether it's at 2, 10, or 50 ug/mL of dox. This is important because it shows that the percent of cells with nuclear expression decreases regardless of how high or low TDP43 expression is, indicating that any level of TDP43 expression will eventually cause a shift away from the nucleus. This is expected because as cells age TDP43 mislocalizes to the cytoplasm where it becomes toxic and forms aggregates. This leads into our next observation that as the cells age we see an increase in the percent of mother cells with foci (Fig. 9). At 24 and 44 hours (Fig. 8B) we see a significant increase in the percent of mothers with foci compared to at 8 hours with higher TDP43 expression (Fig. 8A), indicating that foci formation is a characteristic of age. Interestingly, we see that there is a greater percent of mothers with foci at later time points like 67 hours when TDP43 expression is lower (Fig. 9B). The opposite is true when we look at higher TDP43 expression where the percent of cells with foci is higher at earlier time points and then converts exclusively to nuclear expression (Fig. 9A). This is because as cells age TDP43 mislocalizes from the nucleus to the cytoplasm, but in cells with higher TDP43 expression the aggregates form sooner and become toxic. This is why we don't see mother cells at later time points at higher TDP43 expression forming foci because they die before we can observe protein aggregation. More data needs to be collected to confirm these observations.

C. Future directions

In the future replicates of the growth assays and microscopy need to be done so that we can get a larger sample size and more accurate data. The optimal concentration of dox also needs to be confirmed by repeating those experiments. Performing these experiments with a mutant strain of TDP43 that has a mutation in the LCD would be interesting to see. We would expect that if the mutation disrupts the function of the LCD then it might re-direct TDP43 mislocalization to the cytoplasm, as the LCD is thought to be a driving force behind this [8]. Another thing that might be interesting is looking at the effect of the addition of rapamycin to this system, because it's been known to rescue TDP43 mislocalization by reducing C-terminal fragment aggregation [9]. The inhibition of Dbr1 (an RNA lariat debranching enzyme) [10] and eIF2a-phosphorylation [11] have also been seen to rescue TDP43 toxicity, which would be interesting to test with this system.

REFERENCES

1. Steinkraus, K.A., Kaeberlein, M., & Kennedy, B.K. (2008). Replicative Aging in Yeast: The Means to the End. *Annu Rev Cell Dev Biol*. 24: 29-54. doi: 10. 1146/annurev.cellbio.23.090506. 123509.

2. Lindstrom, Derek L., & Gottschling, Daniel E. (2009). The Mother Enrichment Program: A Genetic System for Facile Replicative Life Span Analysis in *Saccharomyces cerevisiae*. *Genetics*. 183(2): 413-422. doi: 10. 1534/genetics. 109. 106229.

3. Johnson, Brian S., McCaffery, J. Michael, Lindquist, Susan, & Gitler, Aaron D. (2008). A yeast TDP43 proteinopathy model: Exploring the molecular determinants of TDP43 aggregation and cellular toxicity. *PNAS*. 105(17): 6439-6444. doi: 10. 1073/pnas.0802082105.

4. Dobra, Ioana, Pankivskyi, Serhii, Samsonova, Anastasiia, Pastre, David, & Hamon, Loic. (2018). Relation Between Stress Granules and Cytoplasmic Aggregates Linked to Neurodegenerative Diseases. *Current Neurology and Neuroscience Reports*. 18(107). doi: 10. 1007/s11910-018-0914-7.

5. Mackenzie, Ian R.A., & Rademakers, Rosa. (2008). The Role of TDP43 in Amyotrophic Lateral Sclerosis and Frontotemporal Dementia. *Curr Opin Neurol*. 21(6): 693-700. doi: 10. 1097/WCO. 0b013e3283168d1d.

6. Gossen, M., & Bujard, H. (1992). Tight Control of Gene Expression in Mammalian Cells by Tetracycline-Responsive Promoters. *Proc Natl Acad Sci USA*. 89(12): 5547-5551. doi: 10. 1073/pnas.89. 12.5547.

7. Gietz, Daniel R., & Schiestl, Robert H. (2014). High Efficiency Yeast Transformation Using the LiAc/SS carrier DNA/PEG Method. Nature Protocols. 2(1): 31-33. doi:10. 1038/nprot.2007.13.

8. Babinchak, Michael W., Haider, Raza, Dumm, Benjamin K., Sarkar, Prottusha, Surewicz, Krystyna, Choi, Jin-Kyu, & Surewicz, Witold K. (2019). Phase Separation Promotes Aggregation of TDP-43. *The Journal of Biological Chemistry*. 294: 6306-6317. doi: 10. 1074/jbc.RA118.007222.

9. Caccamo, Antonella, Majumder, Smita, Deng, Janice J., Bai, Yidong, Thornton, Fiona B., & Oddo, Salvatore. (2009). Rapamycin Rescues TDP-43 Mislocalization and the Associated Low Molecular Mass Neurofilament Instability. *J Biol Chem*. 284(40): 27416-27424. doi: 10. 1074/jbc.M109.031278.

10. Armakola, Maria, Higgins, Matthew J., Figley, Matthew D., Barmada, Sami J., Scarborough, Emily A., Diaz, Zamia, Fang, Xiaodong, Shorter, James, Krogan, Nevan J., Finkbeiner, Steven, Farese, Robert V., & Gitler, Aaron D. Inhibition of RNA Lariat

Debranching Enzyme Suppresses TDP-43 Toxicity in ALS Disease Models. (2012). *Nat Genet*. 44(12): 1302-1309. doi: 10.1038/ng.2434.

11. Kim, Hyung-Jun, Raphael, Alya R., LaDow, Eva S., McGurk, Leeanne, Weber, Ross, Trojanowski, John Q., Lee, Virginia M.-Y., Finkbeiner, Steven, Gitler, Aaron D., & Bonini, Nancy M. (2013). Therapeutic modulation of eIF2a-phosphorylation rescues TDP-43 toxicity in amyotrophic lateral sclerosis disease models. *Nat Genet*. 46(2): 152-160. doi: 10.1038/ng.2853.

12. Wasko, Brian M., & Kaeberlein, Matt. (2014). Yeast Replicative Aging: A Paradigm for Defining Conserved Longevity Interventions. *FEMS Yeast* Res. 14(1):148-159. doi: 10.1111/1567-1364.12104.