Designing a Novel Cell Aging Model in *Saccharomyces cerevisiae*

Introduction

As organisms continue to age, the number of age related diseases such as cancer and neurodegenerative diseases increases. By stopping or stalling the aging process, the onset of these diseases will be slowed thus allowing people to live a longer, healthier life. To study the aging process, a novel method to study post mitotic cells needs to be designed. Post mitotic cells, such as neurons, model the aging process because these cells are no longer dividing. The cells that will be used to construct this method are yeast because yeast are a single eukaryotic celled model organism that has similar cell machinery compared to the human cell. This makes yeast, specifically *Saccharomyces cerevisiae,* a good cellular aging organism. The current methods that describe the lifespan of yeast are Replicative life span (RLS) and Chronological life span (CLS). These two methods are not sufficient to study the aging process because the yeast cells used in the RLS are mitotic cells, meaning these cells are still dividing. The yeast cells used in CLS are post mitotic cells, but they do not have an unlimited nutrient supply unlike post mitotic cells in humans (Postnikoff & Harkness et al., 2014). For these reasons, neither the RLS or CLS should be used to study the aging process in humans, which is why a novel method needs to be invented.

Our proposed aging model will use the temperature-sensitive CDC28-4 strain of *Saccharomyces cerevisiae* which will be arrested in the cell cycle (post mitotic) and be provided with an unlimited source of nutrients by replacing the media every other day. We will measure the amount of time it takes for the yeast to die. Then, known lifespan-extending compounds such as dinitrophenol, metformin, rapamycin, resveratrol, sperimidine, will be added to test if these compounds will extend the life span of the yeast (Madeo et al., 2019). The CDC28 gene encodes for a cyclin-dependent protein kinase that needs to be present for the cell to enter the S phase of

the cell cycle (Lew & Reed et al., 1993). At 37°C the temperature-sensitive mutant cyclin dependent kinase denatures and does not allow the yeast cell to enter into the S phase and begin cell divisions. Cells arrested in the G1 phase act similarly to G0 phase post mitotic cells which is why these cells will be used in the proposed model. By changing the temperature of the solution the yeast cells are grown in from 25°C to 37°C, the cell cycle can be arrested creating post mitotic cells.

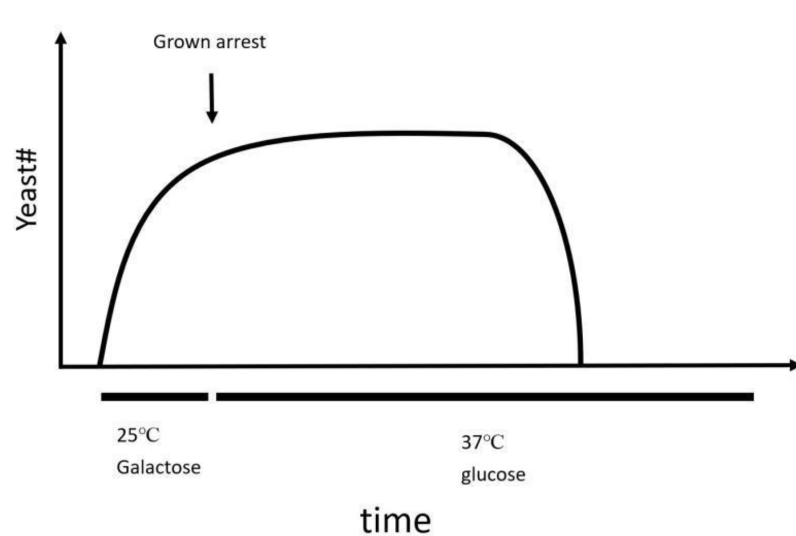

Figure 1. Novel model of yeast growth. The temperature will be switched from 25°C to 37°C and the media will be switched from YP-Gal (Galactose) to YPD (Glucose) once the yeast are half saturated. The yeast's absorbance will be taken at 600 nm at 23° C and in the presence of galactose to determine its saturation level.

Figure 2. The petri dishes shown above contain the CDC28 temperature-sensitive mutant yeast strain grown at 25°C (top) and 37°C (bottom).

To ensure there is no revertant cell from the CDC28 temperature sensitive strain, we will implement the second switch to the CDC28 temperature sensitive yeast by using CRISPR-Cas9 to replace the endogenous promoter sequence to the GAL1 promoter. The GAL1 promoter controls whether the CDC 28 gene is transcribed therefore controlling the cell cycle of the yeast. The GAL1 promoter is activated with galactose and inactivated with glucose. Galactose will bind to the repressor protein on the promoter sequence and change the conformation of the repressor protein allowing the repressor protein to be removed. The appropriate DNA transcription molecules will be recruited to transcribe CDC 28, make the cyclin-dependent protein kinase, thus beginning the cell cycle (O'Connor 1). The media the yeast survives on will be changed when the yeast are saturated by centrifuging the yeast and changing the media from YP-Gal (Galactose) to YPD (Glucose). After changing the temperature and changing the media, the yeast will be arrested in the cell cycle and will be monitored by doing daily serial dilutions to calculate

the concentration of the yeast in the flask. The amount of time it takes the yeast to die alone and with the anti aging drugs will be recorded.

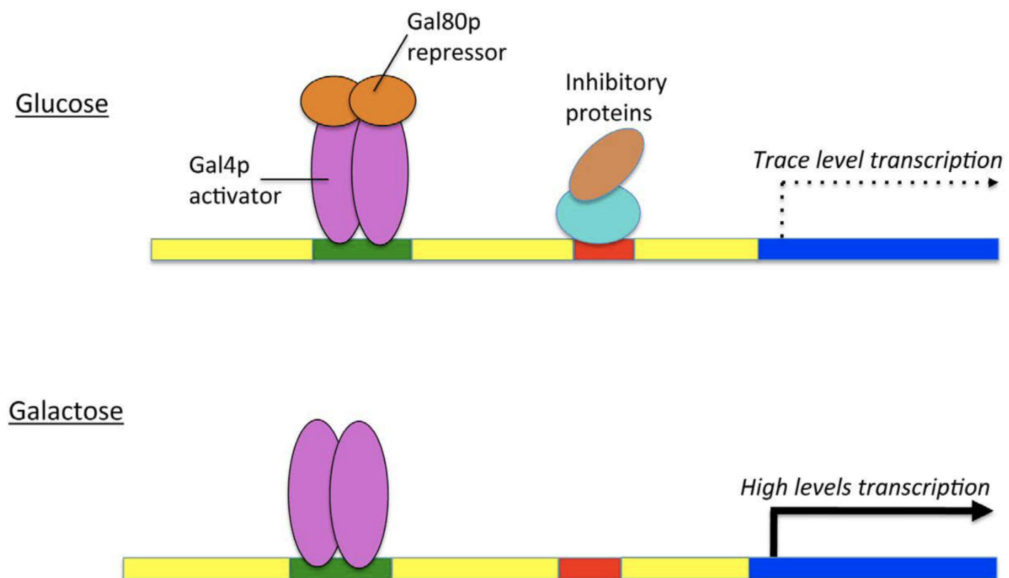

Figure 3. The figure above shows how the GAL1 promoter functions with glucose and galactose. With glucose, repressors are not removed and no transcription of the CDC28 gene occurs. With galactose, the repressors are not activated allowing for transcription of CDC28 to occur (O'Connor 1).

Procedure and Materials

Yeast strain and growing condition

The *Saccharomyces cerevisiae* yeast with the CDC28-4 strain will be used for these experiments. The yeast will be grown in autoclaved YPD media. The media will be made by combining 0.5g of yeast extract, 1g of peptone, and 1g of dextrose per 50mL in a 125mL Erlenmeyer flask. The yeast will be incubated at 25℃ in a 150-RPM incu shaker for two days.

Inserting the GAL1 Promoter with CRISPR-Cas 9

To replace the endogenous promoter with the GAL1 promoter into the CDC28 temperature-sensitive yeast, CRISPR-Cas9 will be used. The pML104 plasmid will be used to carry the CRISPR-Cas9 machinery to the yeast. The specific guide RNA will be designed from the US Department of Health and Human Services protocol (Laughery & Wyrick et al., 2019) and implemented into the pML104 plasmid. This process will ensure the Cas9 protein complex can correctly identify the target cutting site of the yeast's DNA. After the guide RNA sequence is verified, it will be implemented into the pML104 plasmid by DNA sequencing. Yeast cotransformation of both pML104 and the repair template plasmid will be performed. As the Cas9 protein complex creates the double stranded break at the targeted site, homologous recombination from the repair template will fix the double stranded break and replace the endogenous promoter with the Gal1 promoter. After cotransformation, the yeast will be spread onto Ura-galactose plates to verify the plasmid has been successfully transformed and correctly edited by the Cas9 complex. One of the colonies will be chosen and grown with Ura-galactose liquid media. The genomic DNA will be extracted from the yeast and PCR will be performed to

verify the Gal1 promoter is implemented. A lifespan assay will be performed to verify the novel model by using different lifespan-extending compounds.

Timeline

June-July: Designing and implementing guide RNA sequence to pML104

August-September: Yeast cotransformation

October-December: Yeast breeding and selection

December-May: Validating yeast cells in different conditions

Item	**Quantity**	**Cost**
pML104 plasmid	1	$75.00
Frozen-EZ Yeast Transformation II Kit	1	$104.00
ZymoPURE Plasmid Miniprep Kit(100)	1	$169.00
ampicillin (25g)	1	$183.00
Gal1 promoter Repair template plasmid	1	$124.50
sgRNA oligonucliotide	1	$84.00
Galactose (1kg)	1	$240.00
dam-/dcm- Competent E. coli	1	198.00
	Total	$1177.5

References

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