DECIPHERING THE FUNCTION OF *YDL167c* IN *SACCHAROMYCES CEREVISIAE*

Taegan Eiler, Adam Haynes

Abstract

YDL167c is a gene of unknown function. *Saccharomyces cerevisiae* was used as a model organism in an attempt to determine the function of *YDL167c*. Spot assays were performed on WT and the *YDL167c* knockout strain under three stress conditions to determine the growth of the WT strain and PCR confirmed knockout *YDL167c* strains This experiment worked by knocking out *YDL167c* and replacing it with *URA3*. Based on the results from various bioinformatics resources (see methods), *YDL167c* may function in RNA binding, the transport of RNA, and or ribosomal assembly while being located in a stress granule found in the cytoplasm.

Key words: Deciphering Function, Knock-out gene, YDL167c, Saccharomyces cerevisiae, GUF, GUF-gene of unknown function

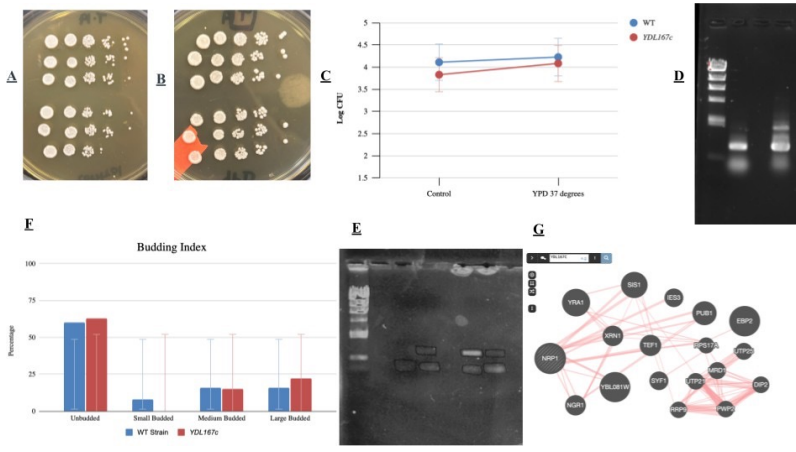

Figure 1: ***A**,YPD at 30°C (control). The top three rows represent the wild type strain and the bottom three rows represent the YDL167c gene deletion strain. Both strains were placed using a spot assay protocol. **B**, YPD at 37°C (experimental). The top three rows represent the wild type strain and the bottom three represent the YDL167c gene deletion strain. Both strains were placed using a spot assay protocol. **C**, interaction plot of wild type yeast concentration vs. YDL167c gene deletion concentration obtained by quantitation of spot assay results. **D**, agarose gel of PCR products from deletion PCR procedure. This was used to determine if a deletion PCR product had been created. The lanes are as follows; Lane 1: Promega Lambda HindIII DNA standard, Lane 2: Taegan Eiler's sample, Lane 3: empty, Lane 4: Adam Haynes' sample. **E**, agarose gel of confirmation PCR products. This was used to determine accurate deletion of the gene YDL167c from yeast. The lanes are as follows; Lane 1: Promega Lambda HindIII DNA standard, Lane 2: empty, Lane 3: Taegan Eiler's sample A, Lane 4: TE sample B, Lane 5: empty, Lane 6: Adam Haynes' sample A, Lane 7: AH sample B. **F**, bar graph displaying results from the morphological analysis of log-phase yeast cells; 100 cells of each strain were measured. **G**, screenshot of a GeneMania produced graph showing physical interactions between YDL167c (NRP1) and other genes. Examples include various ribosomal and RNA binding proteins such as NGR1, PUB1, and RPS17A.*

Description

Saccharomyces cerevisiae is a eukaryotic model organism. While *Saccharomyces cerveisiae*'s genome has been sequenced, there are many genes still remaining that have unknown functions. *YDL167c* is a gene of unknown function located in the *Saccharomyces cerevisiae* genome. This experiment attempts to decipher the function of *YDL167c* using a combination of bioinformatics and wet lab methods that disrupt the *YDL167c* gene and replace it with *URA3*, a gene of well known function. After all of the bioinformatics information was compiled, we hypothesized that *YDL167c* is located in the cytoplasm close to the nucleus with a high probability of being involved in either the transport of RNA or the assembly of the ribosome. The wet lab research conducted showed successful disruption of *YDL167c* with *URA3*. Through spot assays and morphological analysis, both WT and *YDL167c* deletion strains grew at the same rate, suggesting that mitosis was not affected. We chose the stress conditions based on the bioinformatics that showed *YDL167c* has a possible impact on ribosomal stress granules which could be linked to *YDL167c*'s hypothesized function of being involved in RNA assembly, binding, or

processing. The function of *YDL167c* in the *Saccharomyces cerevisiae* genome was not found, but the results state that possible association with RNA binding, ribosome assembly, or RNA processing could be the function of *YDL167c.* Further testing and repeats of this experiment are needed.

Methods

Bioinformatics

Multiple bioinformatics databases were analyzed over the course of a few weeks. The Blast database was used to investigate the function and sequence of *YDL167c*. The *Saccharomyces* Genome database was used to investigate known information of the *YDL167c* gene. Conserved Domain Database, Pfam, and Protein Database were all used to determine the functional groups/protein domains associated with *YDL167c*, which produced multiple results relating to ribosomal assembly, transportation and RNA binding. Genemania was used to look for large sets of functional data between *YDL167c* and other proteins, genes, or subunits. Genemania also analyzed coexpression, physical and genetic interactions, shared protein domains, co-localization, pathways, and predicted relevant genes and functions in correlation with *YDL167c* (Figure 1G). SPELL (Serial Pattern of Expression Levels Locator) database was used to look for commonalities in microarray expression. Through Genemania and SPELL, interactions with other proteins/genes helped to localize the cellular process and pathway of *YDL167c*; both of which referenced RNA. Transmembrane Helices Hidden Markov Models (TMHMM) determined that no transmembrane helices were present. TargetP and Yeast Localization database predicted, based on GFP protein fusion, of localization in the cytoplasm. Phobius predicted that the gene *YDL167c* would not be a transmembrane protein. SignalP did not predict the presence of any signal peptides for processing through the endoplasmic reticulum. PSORT II predicted that multiple proteins play a part in *YDL167c*. The

most notable would be the possible location and function of *YDL167c*; in the nucleus and somehow involved in transcription or translation. LoQAtE (The localization and quantitation atlas of the yeast proteome) predicts localization to the cytosol when grown under SD media.

Gene Deletion PCR

In this procedure, a gene deletion polymerase chain reaction product was completed in an attempt to disrupt *YDL167c* and amplify the replacement product containing *URA3*. The forward and reverse primers used were both 60 base pairs in length, reading up and down stream of the targeted gene to find the complementary base pairs to match their own sequence. The purpose was to amplify the *URA3* gene to increase the likelihood of natural recombination in place of *YDL167c*. Both primers were placed into a PCR tube with the pRS406 template DNA and then ran in the thermocycler according to standard amplification conditions (Nekamasu et al 2022). After the reaction was complete, the *Dpn*I restriction enzyme was added and the PCR mix was allowed to incubate for 60 minutes at 37°C in order to eliminate background transformants of the plasmid DNA. *Dpn*I cuts the plasmid, but will not react with the PCR product generated in the reaction. The sample was then incubated and stored at -20°C. The PCR reaction showed successful amplification of *URA3* after gel electrophoresis (Figure 1D).

Transformation of yeast

This portion of the procedure was completed with a control sample and the PCR product from the gene deletion PCR. Both samples are mixed with PEG 3350, ssDNA, and lithium acetate in microfuge tubes before being put through multiple steps in an attempt to transform normal yeast DNA cells with the deletion PCR product (Gietz, Schiestl 2007). The transformation samples were inoculated onto SDC-URA plates and incubated at 30°C for four days following the

transformation. The SDC-URA plates were made from agar that was lacking uracil, which means that only the yeast containing *URA3* would be able to grow. The negative sample contained water, so there was no *URA3* gene present to promote growth. The positive sample contained the *URA3* gene deletion PCR product, which grew successfully. This pattern of growth suggests that the transformation was successful at disrupting *YDL167c* and replacing it with the *URA3* gene to obtain 121 transformant yeast colonies.

Confirmation PCR

The goal of confirmation PCR is to identify if the knockout of *YDL167c* with *URA3* was successful or not. This major section can be broken down into three smaller parts; isolating DNA from a single yeast colony, streaking the presumed deletion colonies, and running the PCR confirmation procedure. In order to isolate the DNA from the transformed yeast colonies, half of a single colony was taken from the agar plate grown in the transformation process mentioned above. The sample went through a handful of steps in order to break down the cell and denature the DNA, making it more accessible to further testing (Looke et al 2011). The other half of the colony was transferred and streaked onto a new SD-URA agar plate that was incubated at 30°C for four days. The final step was to run the newly isolated DNA through the thermocycler to achieve a PCR product. The DNA was mixed with the appropriate primers before being run in the thermocycler. These primers read the yeast DNA to reveal if the knockout of *YDL167c* and replacement of *URA3* was successful or not. The forward primer used was located upstream of *YDL167c*. The reverse primer used was JKS003, located within *URA3*. The product's size was expected to be approximately 950 base pairs long, made up of 800 base pairs of *URA3* and 120 base pairs upstream of the start of the gene. After this, the product was run on an agarose gel (Figure 1E) to determine that the PCR reaction was successful at disrupting *YDL167c* and replacing it

with *URA3*. We were successful as gel electrophoresis produced a band around 900 in the expected lane while the standard expected range was between 750-950 base pairs (Figure 1E).

Spot Assay Analysis

A spot assay was completed to compare growth of the wild-type and *YDL167c* mutant strains.. Three replicas for the wild-type strain and the *YDL167c* deletion strain were spotted onto each of our four plates. Before the plates were spotted, a 10 fold serial dilution had to be completed. The start point was a culture of yeast at 0.1 OD600 and four dilutions were made. 2µl of each dilution were then spotted onto the four agar plates before being incubated at either 30°C or 37°C for approximately 72 hours. After the plates were taken out of the incubator, they were photographed and the single colonies were counted to figure the approximate concentration of the original suspension (Figure 1A-C). This whole process was completed twice in an attempt to increase the precision of the data.

Morphological Analysis

A morphological analysis was completed to visually assess the percentage of cells in each phase of budding; not budding, small budding, medium budding, and large budding. Both of the cultures used for this step were collected during the log phase to assure that there would be plenty of dividing cells to observe. The samples were mixed with phosphate buffer and formaldehyde in between being centrifuged and sonicated to isolate individual cells for better viewing. Once the cultures have completed the isolation process, both were mounted onto their own glass slide and viewed under the microscope. An image was captured and imported into the ImageJ program to be analyzed. In total, 100 cells were measured and recorded from both the wild-type and *YDL167c* deleted strains. The cell measurements were used to determine where in the budding process the cells were (Figure 1F).

Reagents

Table 1: Yeast Strains used in this study

Yeast Strain	Genotype	Source
Wild-type strain	BY4741	Provided by instructor
Deletion strain	*ydl167c::URA3*	Created in this study

Table 2: Additional components used

Components	Genotype	Source
Plasmid	pRS406	Provided by instructor
Forward primer in gene deletion PCR	116	Provided by instructor
Forward primer in confirmation PCR	JKS136	Provided by instructor
Reverse primer in gene deletion PCR	117	Provided by instructor
Reverse primer in confirmation PCR	JKS003	Provided by instructor

Table 3: Media used in this study

Media	Strains Tested	Stress Conditions
YPD at 30°C	WT and *ydl167c*	Control plate
YPD at 37°C	WT and *ydl167c*	Heat shock
YPD plus sorbitol at 30°C	WT and *ydl167c*	Osmotic shock
YPG at 30°C	WT and *ydl167c*	Glucose starvation

Statistical Analysis

Table 4 contains the various p-values gathered from the colony forming unit quantification from both trials. P-values measure the probability of if the observed results were due to chance or not. If there is a high probability that the results are due to chance, then it is likely that the null hypothesis is correct. If there is a high probability that the results are not due to chance, then it is likely that the null hypothesis is incorrect. If the value is less than 0.10, then there is some evidence against the hypothesis. If the value is greater than 0.10, then there is a

good chance that the hypothesis is correct.

In trial 1, the heat shock plate and +sorbitol plate show a much greater chance that the growth was affected by the environment and not the deletion of *YDL167c*. The YPG plate does not show any difference in growth between the two strains when comparing the control to the stress plate. In trial 2, the YPG plate shows that there was a much greater chance that the growth was affected by the deletion of *YDL167c* and not the plate condition used. The heat shock plate shows a slight increase in likelihood that the growth was affected by deletion and not the plate condition. The +sorbitol plate does not show any difference in growth between the two analytical factors. ■

Table 4: P-Values from ANOVA analysis

P-values gathered from ANOVA analysis						
Conditions in comparison to YPD control	Trial 1			Trial 2		
	Genotype	Environment	GxE	Genotype	Environment	GxE
YPD at 37°C	0.924100	0.049363	0.638362	0.087468	0.126120	0.542053
YPD plus sorbitol at 30°C	0.548736	0.147505	0.988688	0.514676	0.537471	0.130217
YPG at 30°C	0.600408	0.604552	0.356328	0.090973	0.566632	0.712707

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