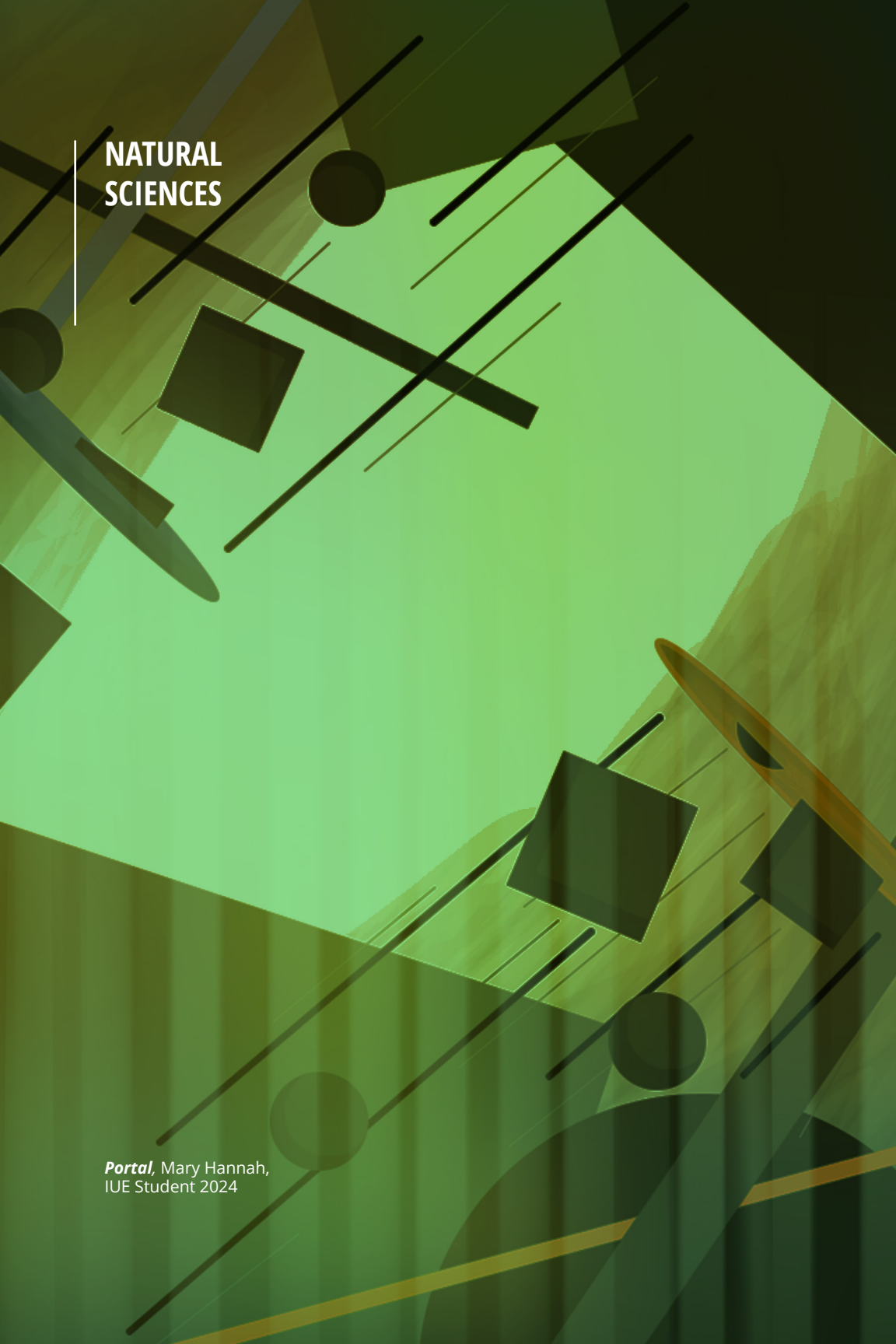
NATURAL SCIENCES



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IUE Student 2024

YDL167c GENE FUNCTION IN *S. CEREVISIAE*

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Abstract

Saccharomyces cerevisiae, a budding yeast, is a single-celled eukaryotic organism used to study molecular and cellular processes and pathways. Although the DNA sequence of all yeast genes is known, the function of over 1,000 yeast genes is unknown. In this study, the potential function of *YDL167c* was examined using bioinformatics and wet lab experiments. Using the DNA and protein sequence of *YDL167c* , biological databases, such as "Gene Mania", were searched to find likely protein domains and cellular localization information. The results of these investigations showed that the gene *YDL167c* had multiple connections with the genes NGR1 and EBP2. The function of NGR1 is RNA binding, and it negatively regulates growth rate. EBP2 localizes to nucleolus and is needed for 25s rRNA maturation and 60s ribosomal subunit assembly. This shows the *YDL167c* gene has many connections to proteins that are involved in RNA binding. Next, a yeast strain bearing a knock-out of *YDL167c* was generated using a PCR-based method. Successful disruption of *YDL167c* with the *URA3* gene was verified using a second PCR reaction. Finally, spot assays and morphological analyses were performed to compare the growth of *YDL167c* yeast to the wild-type strain. The results from the spot assay did not show more sensitivity with the knockout *YDL167c* deletion strain in the stress conditions: YPD 37°C high temperature, YPD plus 4% ethanol at 30°C, and YPD at 30°C compared to the wild-type. The *YDL167c* deletion strain demonstrated a slight increase in sensitivity to the stress condition, YPD plus 0.5 M NaCl at 30°C, compared to wild-type.

Key words: *Saccharomyces cerevisiae, budding yeast, YDL167C , bioinformatics, wet lab experiments, GeneMania, EBP2 , NGR1 , PCR-based method, RNA binding, knock-out gene, deletion strain, stress condition, negatively regulates growth rate*

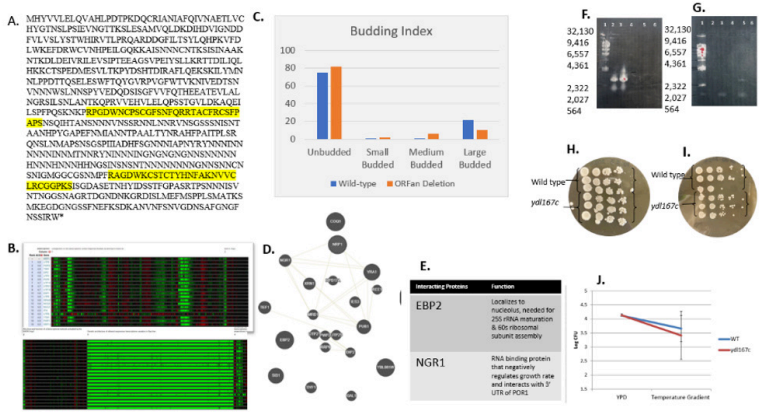


Figure 1:

A, Protein sequence of YDL167c gene with important protein domains highlighted. Both domains highlighted are zinc finger domains in Ran binding protein and others. B, Shows NRP1(YDL167C gene) consistently responds to transcriptomic stress response and inositol pyrophosphates; obtained from SPELL database. C, Bar graph of results from morphological analysis. D, Shows the interaction of NRP1 and EBP2 proteins with YDL167C gene. Acquired from Gene Mania database. E, Proteins that interact with YDL167C, acquired from Gene Mania database. F, Agarose gel electrophoresis used to check the presence of deletion PCR. Lanes: 1. Lambda HindIII standard, 2. first sample, 3. second sample. G, Agarose gel electrophoresis used to check the presence of confirmation PCR. Labeled left to right, 1. Lambda HindIII standard, 2. N/A, 3. first sample, 4. N/A, 5. second sample. H, YPD (no stress) plate at 30° C and incubated for 2 days. The top 3 rows are wild-type strain, and the bottom 3 rows are ydl167c deletion strain. I, YPD plate, stress condition 37° C high temperature and incubated for 2 days. The top 3 rows are wild-type strain, bottom 3 rows are ydl167c deletion strain. ydl167c deletion strain is more sensitive to high temperature than the wildtype. J, Interaction plot from spot assay analysis results ("Temperature gradient" is YPD at 37° C).

Description

An RNA binding protein is a protein that binds itself to either a single or double stranded RNA and contributes to the formation

of ribonucleoprotein complexes. RNA binding proteins bind within the nucleus but also continue functioning within the cytoplasm.

Bioinformatics evidence showed that *YDL167c* has a potential function in RNA binding. SPELL analysis was utilized to determine GO terms enriched in the genes expressed similarly to *YDL167c* . Genes expressed similarly within *YDL167c* were UTP8 and PWP2 genes. UTP8's function is related to transcription regulation of RNA polymerase I. The function of PWP2 is mRNA-binding. Gene Mania showed multiple relation hits to NRP1 including EBP2, and NGR1 (Figure 1D-1E). NGR1 is an RNA binding protein that negatively affects growth rate. EBP2 localizes to nucleolus, needed for 25s rRNA maturation and 60s ribosomal subunit assembly. However, after conducting wet lab experiments there has been no further evidence to back up the hypothesis of possible functionality in RNA binding. After plating *YDL167c* deletion strain and wild-type on two stress conditions, 4% ethanol and 37°C high temperature, there was no significant difference in sensitivity between the wild-type and the deletion strain. On the stress condition of 0.5 M NaCl, the *YDL167c* deletion strain demonstrated higher sensitivity compared to wild-type. While using ImageJ to perform morphological analysis, the results showed that wild-type had more cells in the later stages of the cell cycle than the deletion strain and the deletion strain had more cells in the earlier stages of the cell cycle compared to the wild-type (Figure 1C).

Future Direction

In the future, more morphological analyses could be performed to confirm the significance of the results found during this experiment. More cells need to be analyzed utilizing ImageJ to confirm the significance of the findings of *YDL167c* deletion strain having more cells in G1 phase (unbudded) compared to wild type.

Methods

Gene Deletion PCR

In this experiment, forward primer (JKS136), reverse primer (JKS003), and template DNA (pRS406) were inserted into a PCR tube that contained buffer, deoxynucleotides, and Taq polymerase. The PCR was cycled, conditions were based off modified protocol from, "Yeast ORFan Gene Project Wet Lab Modules" to create the gene deleted PCR product. At the completion of the cycles 1 µl Dpnl restriction enzyme was added to the tube, and then the tube was incubated for 60 minutes at 37°C. Agarose gel electrophoresis was used to visualize the PCR product (Figure 1F).

Yeast Transformation

2 yeast cell aliquots were thawed then centrifuged. The transformation mixture was prepared by adding PEG, ssDNA, 1M lithium acetate, and 14 µl of deletion PCR product (Gietz and Schiestl 2007). The transformation mixture was added to each cell pellet mix and incubated for 30-45 minutes. Then, lithium acetate/ SDS solution was added. Transformed yeast cells were plated on URA plates and incubated at 30°C for 4 days. Single colonies from -URA transformation plates were used for confirmation PCR.

Confirmation PCR

To confirm the gene ydl167c was successfully deleted and replaced with *URA3* gene, DNA was isolated from transformants and subject to PCR. The template DNA used was isolated from a single yeast colony (Lõoke 2011).The forward primer anneals upstream of the deleted *YDL167c* gene, and the reverse primer binds to the *URA3* gene that takes place of *YDL167c* gene; thus, the PCR product will be amplified if ydl167c gene is disrupted with *URA3*. Standard amplification conditions were used. Then, samples were analyzed by agarose gel electrophoresis (Figure 1G).

Spot Assays

Spot assays were performed by plating serial dilutions of 3 rows of wild-type and 3 rows of the deletion product on each plate of the following stress conditions, YPD plate no stress, YPD plate 37oC high temperature, YPD 4% ethanol, YPD 0.5 M NaCl. These spot assays were performed two times for each stress condition, and all plates were quantified. (See figures 1H-1J.)

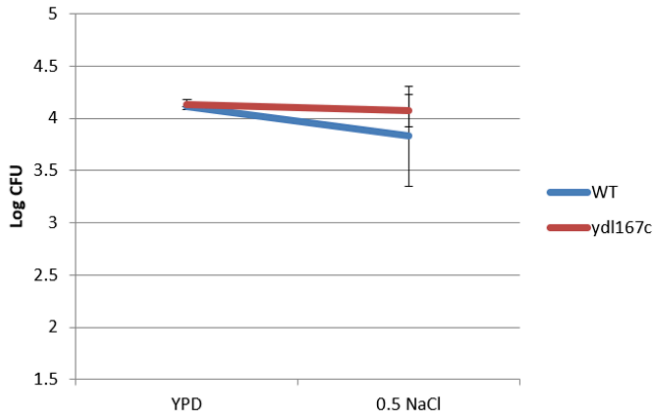
Morphological Analysis

Yeast cells from log phase cultures were prepared and fixed for wildtype and *YDL167c* strain. Cells were imaged under a microscope. Cells were measured utilizing ImageJ; 100 cells were measured for both wildtype and *YDL167c* . (See Figure 1C.)

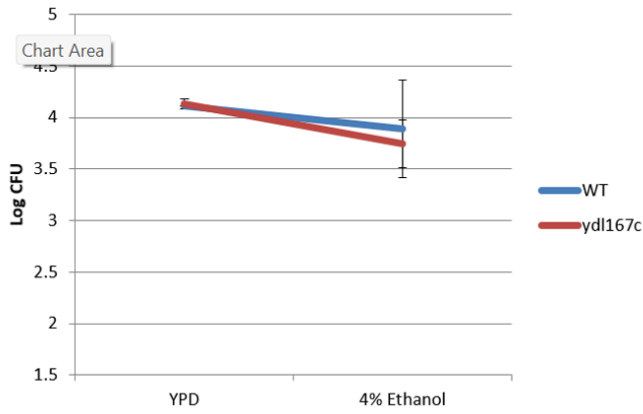
Statistical Analysis

The following graphs show a growth comparison of *YDL167c* deletion strain and wild-type strain under different stress conditions. Two trials of each stress condition were performed.

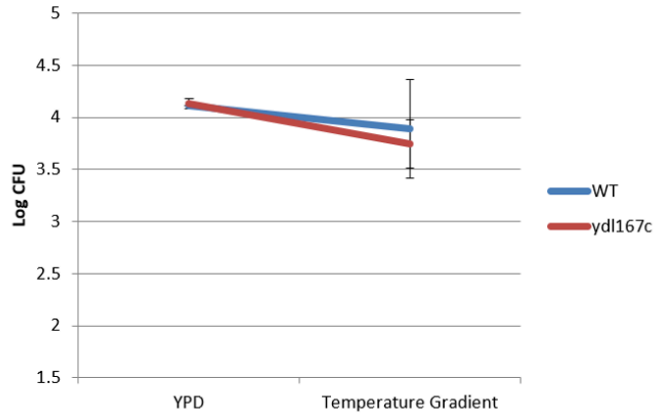
Trial 1:



This graph shows the difference between the wild-type and deletion strain in the stress condition 0.5 M NaCl. The wild-type strain was more sensitive to 0.5 M NaCl compared to ydl167c deletion strain.

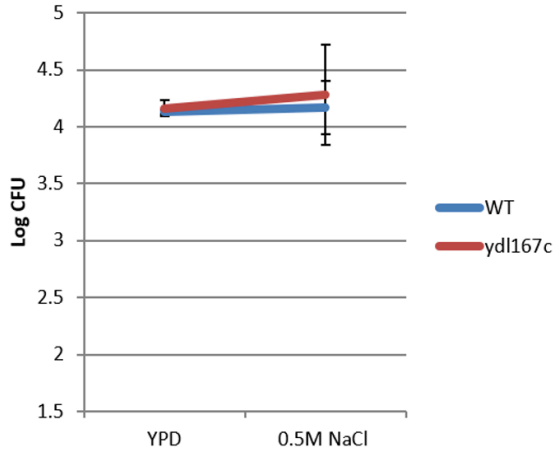


This graph shows the difference between the wild-type and deletion strain in the stress condition 4% ethanol. The *YDL167c* deletion strain was more sensitive to 4% ethanol compared to wild-type strain.

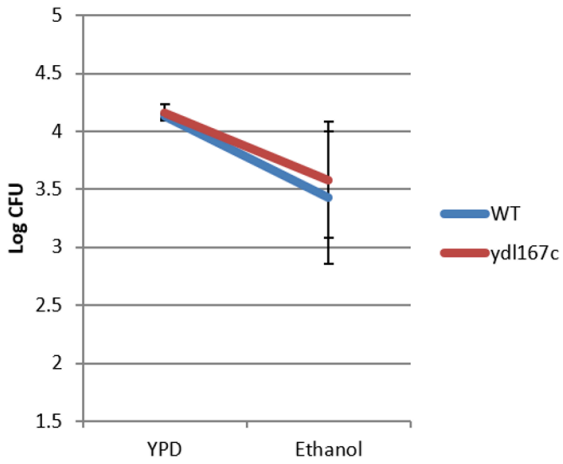


This graph shows the difference between the wild-type and deletion strain in the stress condition 37°C high temperature. The *YDL167c* deletion strain was more sensitive to 37°C high temperature compared to wild-type strain.

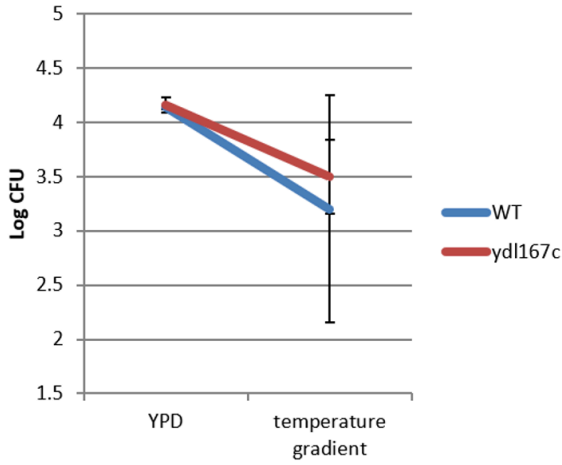
Trial 2:



This graph shows the difference between the wild-type and deletion strain in the stress condition 0.5 M NaCl. The wild- type strain was somewhat more sensitive to 0.5M NaCl compared to *YDL167c* deletion strain.



This graph shows the difference between the wild-type and deletion strain in the stress condition 4% ethanol. There was not a significant difference between the sensitivity of *YDL167c* deletion strain and wild type but both strains were highly sensitive to 4% ethanol.



This graph shows the difference between the wild-type and deletion strain in the stress condition 37°C high temperature. The wild-type strain was more sensitive to 37°C high temperature compared to *YDL167c* deletion strain. ■

Reagents

Table 1: Yeast Strain used in this study

Strain	Genotype	Source
Wild-type strain	BY4741	Provided by instructor
Deletion Strain	*ydl167c:URA3*	Created in this study
Plasmid	*pRS406*	Provided by instructor
Forward primer for confirmation PCR	JKS136	Provided by instructor
Reverse primer for confirmation PCR	JKS003	Provided by instructor
Forward primer for gene deletion PCR	116	Provided by instructor
Reverse primer for gene deletion PCR	117	Provided by instructor

Table 2: Stress Conditions

Medium	Strain Tested	Goal
YPD at 30°C	WT and *ydl167c*	Control Plate
YPD plus 0.5 M NaCl at 30°C	WT and *ydl167c*	Osmotic Stress
YPD at 37°C	WT and *ydl167c*	Heat Shock
YPD plus 4% ethanol at 30°C	WT and *ydl167c*	Oxidative Stress

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Lõoke M, Kristjuhan K, Kristjuhan A. Extraction of genomic DNA from yeasts for PCR-based applications. *Biotechniques*. 2011 May;50(5):325-8. doi: 10.2144/000113672. PMID: 21548894; PMCID: PMC3182553.

Acknowledgements:
Faculty mentor, Dr. Jill Schweitzer and Laboratory manager, Heatherlynn Barrett
Yeast ORFan Gene Project

Funding:
The School of Natural Science and Mathematics at IU East and the Women's Philanthropy Leadership Council of the IU Foundation