Protein Function YJR120W in the *Saccharomyces cerevisiae* Yeast Genome

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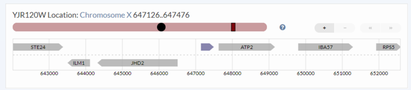
ABSTRACT

Saccharomyces cerevisiae is a yeast that is classified as a model organism due to its ability to be easily bred and maintained. Twenty years of study has produced a sequenced genome; however, ten percent of its gene function remains unknown. One of the genes of unknown function in *Saccharomyces cerevisiae* is *YJR120W*. It has been determined that *YJR120W* is essential for growth in anerobic environments, has decreased expressions of ATP2, impairs respiration, causes defects in sterol uptake, and alters levels and localization of ABS transporters Ausp1 and Pdr11p. High-throughput screening has also indicated a role in Ty3 retro-transposition. Ty3 is a transposon that resembles a retrovirus with long terminal repeats containing open reading frames that function similarly to Gag and Pol proteins. The retroelement integrates upstream of genes transcribed by tRNA and polymerase III; transposition is detected after Ty3 transcription. Translation of the RNA and proteins encoded by the element generates retroviral genes for Gal and Pol proteins, which contain genes for capsid proteins, nucleocapsid proteins, protease, reverse transcriptase, and integrase. On the plasmid, Gal1 is the upstream activating sequence promoter that regulates Ty3 transcription. Transcription on galactose media introduces the plasmid encoded element copy into the RNA. Reverse transcriptase transcribes mRNA to form cDNA. Printing to SCgal-URA induces Ty3 mobility followed by printing to SC-HIS which allows us to examine and select for transposition. The purpose of our study has been to confirm that the mutant deleted for *YJR120W* shows reduced Ty3 transposition. Printing was conducted at three different temperatures—22, 30, 33°C—which increased transposition at 22°C. Spot assays assessed growth by comparing wild type to mutant. On glucose dropout, growth appeared to be the same, but on galactose drop out the mutant growth was decreased; galactose rich media growth was equal. Overall the deletion showed decreased transposition, but also showed decreased growth on galactose. Future research will be conducted to discern if the decreased transposition is due to decreased growth, due to the results on galactose drop out media.

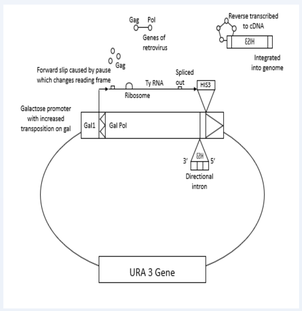
INTRODUCTION

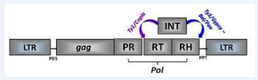
- *Saccharomyces cerevisiae* is a yeast that can be classified as a model organism
  - First complete DNA sequence of a eukaryotic genome
  - Model organism that has been widely studied, is easy to maintain and breed, and is cost effective
  - Useful for interpreting and understanding human biology and DNA sequences
  - Commonly found in baking and brewing yeast
- *YJR120W* is a protein of unknown function in the yeast genome
  - Essential for growth in anaerobic environments
  - Causes decreased expression of ATP2
  - Impairs respiration
  - Causes defects in sterol uptake
  - Alters levels and localization of ABS transporters Ausp1 and Pdr11p
  - Reduced Ty3 transposition in YJR120W deletion
- Location of YJR120W
  - Relatively short protein length of 116 amino acids



- Ty3 is a retroelement that gives insight into retroviruses
  - Specific integration upstream of genes transcribed by RNA polymerase III and tRNA
  - On the plasmid, Gal1 promoter is the activating sequence that regulates Ty3
  - Transposition is detected after Ty3 transcription
  - Translation of RNA and encoded proteins are Gag and Pol, which encode for capsid proteins, nucleocapsid protein, protease, reverse transcriptase, and integrase
  - As noted in the high throughput screening, we are testing levels of transposition

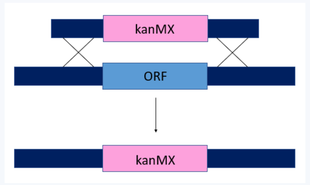
•Ty3 lifecycle and structure





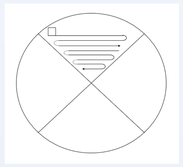
RESULTS

- **Gene Deletion**
  - Deleting the ORF was necessary to assess the phenotype. This was generated by using PCR to replace the gene. The genomic DNA was isolated from a colony of yeast, and then PCR was performed using a primer to kanMX and a primer upstream of the deleted ORF.

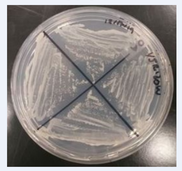


- **PCR**
  - A 50uL reaction was prepared to undergo PCR. 10uL of buffer (5x concentration), 5.0uL of dNTPs (2mM), 1.0uL of both JK859/858 forward and reverse primers (10mM), 0.25uL of 1 TAQ polymerase (Oct. 15th), 1uL of template DNA PRS 400 (2ng per uL), and 33uL of milliQ water which was mixed by pipetting up and down. The solution was warmed for a few minutes in the PCR machine before adding the 1TAQ to preheat. The sample was heated at 94°C for 15 minutes, and then denatured at 94°C for 2 minutes. The cycle included rounds of amplification for denaturing and annealing before running at 4°C until it was removed. Adjustments were made to the sample by trying three different 1TAQ to ensure that product was present, the most successful being the Oct. 15th. Annealing temperatures were also adjusted to achieve a single band of product.
  - PCR was proved unsuccessful when there was no product present on the gel. The deletion strain was ordered from GE Healthcare/Dharmacon.
- **Gel Electrophoresis**
  - Gel electrophoresis was done to confirm that there was PCR product present which indicated that the deletion was successful.
  - 14uL of sample comprised of 2uL loading dye (6x), 3uL of PCR sample, and 9uL of water was loaded into a well alongside 5uL of ladder. The gel was run at 70V for 1 hour. A band is expected to appear at 1.5kbp.

  - A faint band appeared at the 1.5kbp size. The PCR and gel confirmed that the deletion we purchased was the correct one.
- **Transformation with pGTy3mHIS3AI Plasmid**
  - The deletion strain was transformed with pGTy3mHIS3AI so that it could be assayed for transposition. Single colony purification was done to isolate colonies from the deletion to use in further experiments.
  - The transformants were plated in four quadrants on SC-URA using a wooden stick with a pen tip sized amount of cells from four different colonies. A square was smeared in the top left quadrant. A new stick was used to zig-zag smear in the top of the quadrant, and the other side of the stick was used to smear to the bottom of the quadrant, effectively isolating an individual colony. The plate was incubated at 30°C.
  - Patches of the colony purification were made on SC-URA medium and the patches were frozen in glycerol at -20°C for storage. Since the yeast was transformed with pGTy3mHIS3AI this allowed it to be assayed for transposition. The *URA3* gene on the plasmid is to select for transformation for the plasmid. These colonies are confirmed to have the plasmid if the *URA3* gene is present, otherwise there would be no growth on a SC-URA plate. Once purified, the patches are ready for transposition.

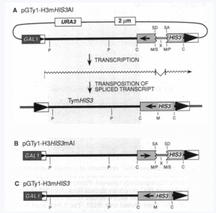


SC-URA

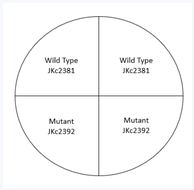


RESULTS

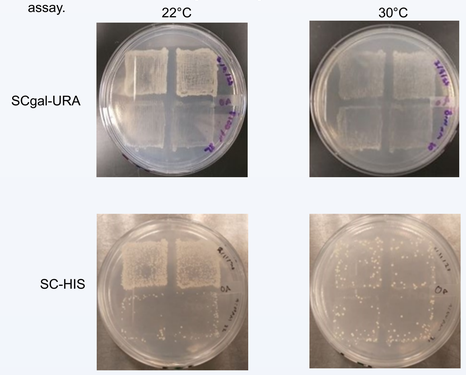
- **Transposition Assay**
  - The transposition assay was conducted to assay the deletion mutant for transposition because high throughput screening has shown that the deletion has decreased transposition.
  - Ty3 is a retroelement with specific integration upstream of genes transcribed by RNA polymerase III and tRNA. On the plasmid, GAL1 is the upstream activating sequence that regulates Ty3. The GAL1 promoters increase transposition on galactose. Before transcription, the mRNA does not yield the protein because the intron is still in place. Plating on SC-URA ensures that the URA3 gene on the plasmid is integrated, otherwise no growth would appear. Plating on SCgal-URA is necessary to induce transposition. Plating on SC-HIS checks for transposition since it has been deleted for HIS. HIS3 protein makes the enzyme required for histidine synthesis and cells grow on media lacking histidine if transposition is successful.



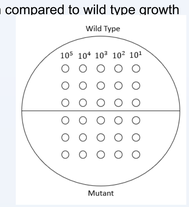
  - Wild type (*JKc2381*) plated on the top 2 squares on the grid. Mutant (*JKc2392*) plated on the bottom of the two squares. SCglu-URA plate incubated at the 30C for 24 hours.
  - Printed onto SCgal-URA and put plates into 22°C, 30°C, and 33°C incubators for 36 hours.
  - Printed onto SC-HIS and placed plates at their respective temperatures. Incubated for 48 hours.



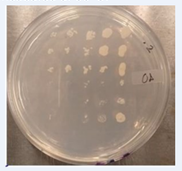
  - The transposition assay showed better transposition at 22°C compared to 30°C in the wild type and decreased mutant transposition compared to the wild type. The mutant also had decreased growth on galactose which was tested with a SPOT assay.



- **SPOT Assay**
  - The SPOT assay was done to assess mutant growth compared to wild type growth on different media at different dilutions.
  - Plated mutant (*JKc2392 209 ORF*) and wild type (*JKc2381 ORF2*) on a YPD plate and incubated at 30°C for 48 hours.
  - Inoculated pen-tip sized amount of cells in 5mL YPD and incubated in 30°C shaker overnight.
  - Diluted 1mL of cells/YPD the next day, 4 hours before plating, into 10mL YPD and put back into the 30°C shaker.
  - Ran spectroscopy of 3mL wild type, mutant, and blank YPD vials with an absorbance of 0.48 to determine that the cells did not need to be diluted more.
  - Pipetted as indicated by the dilutions in the figure to the right for dilutions. Pipetted 2uL from each well onto SC-URA, Scgal-URA, YPD, and minimal media plates. The growth on the plates matches the diagram above. Incubated at 30°C.

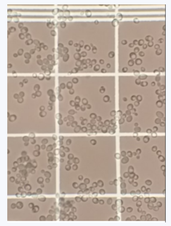


  - On SCgal-URA, mutant growth is decreased compared to the wild type growth. The mutant did not grow well on galactose.

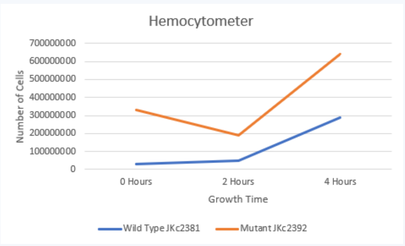


RESULTS

- **Hemocytometer**
  - The hemocytometer was used to compare growth rates in real time and morphology.
  - Inoculated a pen-tip sized amount of cells of both wild type and mutant (JKc2392) into a 10mL YPD and incubated overnight in 30°C shaker.
  - Diluted the cells the next day 1:50 in water. Injected 10uL under the slide until the liquid filled the grid area of the hemocytometer. Viewed under the microscope at the 45x objective and counted cells in the 5x5 grid.
  - LEVY calculation: # x dilution x 10^4



  - The morphology appeared to be the same for both the wild type and the mutant and there are no major differences in growth rates.



CONCLUSIONS

- Transposition assay showed:
  - Better wild type transposition at 22°C compared to 30°C
  - Decreased mutant transposition compared to wild type transposition
  - Decreased mutant growth on galactose
- Spot assay showed:
  - Reduced mutant growth on galactose
- Hemocytometer showed:
  - No difference been wild type and mutant morphology and growth rates
- Overall, the deletion confirmed the results of the high throughput screening that showed decreased transposition. Additionally, the deletion showed decreased growth on galactose. Future research will discern if the decreased transposition is due to decreased growth.

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