Understanding the Gene *Css3* through Analysis of *Css3p* Localization and Interaction in *Saccharomyces cerevisiae*

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Abstract

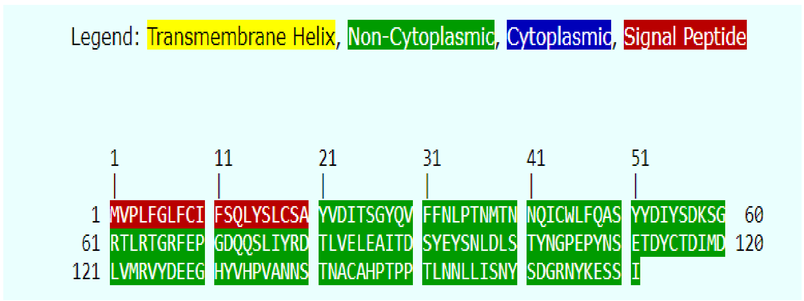
In the quest to understand human genomics, *Saccharomyces cerevisiae* serves as an excellent model organism due to its relative simplicity and quick reproductive turnover. However, despite its advantages, the function of many open reading frames (ORFs) in the species remains undiscovered. This project aims to shine light on the function of one of these ORFs known as Css3, whose deletion has been shown to produce elevated levels of Ty1 retrotransposition; Ty1 is a DNA segment known as a "jumping gene" for its ability to move from one loci to the next. To accomplish this goal, bioinformatics was utilized to yield statistical information on the localization and interactions of the ORF's protein product, Css3p. Bioinformatic analysis indicates that the protein may be non-cytoplasmic, and high-throughput microscopy suggests localization in the cell periphery. Analysis of the physical and genetic interactors with Css3 indicates that Css3p is associated with proteins acting on mRNA decay. Of note, the physical interactor BFR1 is associated with P-bodies, which not only degrade mRNA, but have been shown to promote Ty1 retrotransposition. This data hints that Css3 could potentially influence Ty1 through the regulation of mRNA as well. Future research will involve testing this hypothesis through deletion of the *CSS3* gene and tracking Css3 GFP-fusion proteins via fluorescent microscopy to gather experimental data on Css3p localization. Optimistically, this data may make progress in determining the function of Css3, and perhaps unlock information relating back to the growing understanding of human genetics.

Introduction

- Nearly 1,000 ORFs have been annotated with unknown function in budding yeast, including the gene *Css3*, also known as *YOL159C*. [1]
- Deletion of *Css3* has been linked to increased levels of Ty1 retrotransposition. [2]
- Processing bodies (P-bodies) play a role in mRNA decay and have been shown to enhance Ty1 activity.
- Experimental evidence for yeast genes are stored in the Saccharomyces Genome Database (SGD). Included in this database is information on gene ontology, which is used to characterize genes.
- According to SGD gene ontology, the molecular function and biological processing of *Css3* is unknown, while the cellular component states that the gene's protein product (*Css3p*) localizes to the cell periphery and extracellular region.
- The purpose of this research is to progress in our understanding of function and location of the *Css3p*.

Results- Analysis of Protein Sequence

- First, bioinformatic analysis was used to confirm and further explore findings from the SGD



- **Fig.1- Philius Prediction Sequence Map:** The Prediction Sequence Map indicates that sequence 1-20 of *Css3p* contribute to a signal peptide, while sequences 21-171 are localized in non-cytoplasmic regions of the cell.

Results- Interaction with *Css3*

Table 1: Physical Interactors reported by SGD

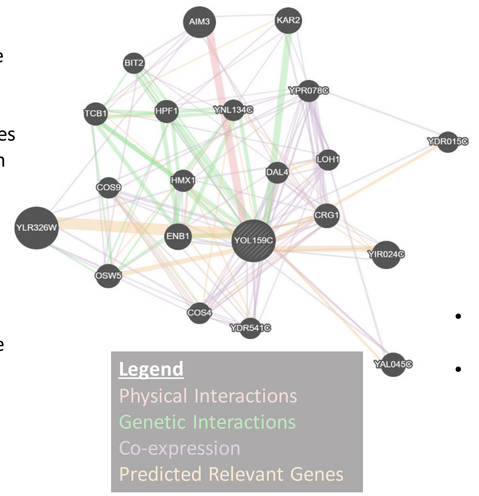
ORF	Function
BFR1	Assists in bringing mRNA to P-bodies
DHH1	Encourages de-capping of mRNA
MPT5	Encourages de-capping and decay of mRNA
SLF1	Potentially involved in mRNA translation

- Physical interactors are genes whose protein products display physical interaction in high throughput experiments.
- Physical interactors with *Css3p* have been reported as in association with mRNA regulation by Saccharomyces Gene Database.[2]
- Since P-bodies are the elements involved in mRNA decay and in Ty1 retrotransposition, this data suggests that *Css3* could additionally play a role in mRNA decay.

Results- Css3 Interaction Network

- GeneMania is a resource used to illustrate the network of a gene's interactions.

Fig.3- Expression & Interaction Network: The network for *Css3* (*YOL159C*) demonstrates the depth of interaction complexity.



- Of note, the GeneMania algorithm identifies the gene *AIM3* as a physical interactor with *Css3,* which was not identified by SGD.
- SGD reports *AIM3* as being a gene of unknown function associated with the mitochondrial genome and localized in the actin cortical patch.
- Additionally, the gene *YLR326W* was a predicted relevant gene to *Css3,* raising the question of whether a relationship truly exists between these genes or if this result was simply due to both genes being of unknown function.

Results- Deletion of CSS3

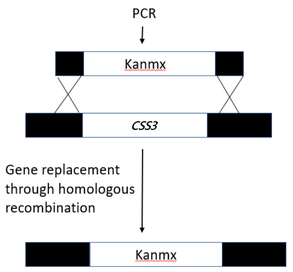


Fig. 3- Mechanism of Css3 replacement: homologous recombination is expected to delete *Css3* and insert *Kanmx* during PCR.

- PCR product was transformed to amplify the gene *Kanmx* which allows yeast to grow on plates treated with G41B. Fig. 4 displays the result of gel electrophoresis used to confirm the gene's successful amplification. The cells were then plated, and the colonies that grew had putatively integrated *Kanmx* in place of *Css3*.
- After bioinformatic analysis of *Css3* and *Css3p*, wet lab procedures were utilized to perform a deletion of *Css3*. The deletion mutant will be used to study Ty1 retrotransposition. In Fig. 3, homologous recombination is expected to delete *Css3* and insert *Kanmx* during PCR.

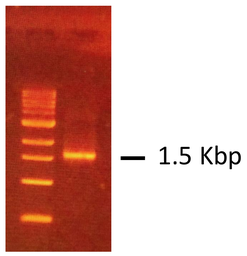


Fig.4- Gel electrophoresis result of deletion: Well #1 contains the ladder and well #2 contains the PCR product. The size of *Kanmx* is approximately 1.6 Kbp, and thus the position of this the band in Well #2 indicates that *Kanmx* was integrated.

Results- Studying the Deletion Phenotype

Transposition Assay

- Once the deletion strain was obtained, a transposition assay was conducted to study Ty1 and Ty3 transposition. This involved transforming some yeast with a URA3 galactose inducible Ty1 element marked with hisAI, and other yeast with this same plasmid but substituted with Ty3 element. Transposition was to be studied on SC-URA glucose, SC-URA galactose, and SC-his media, but the deletion strain displayed growth deficiency on galactose.

Spot Assay

- To further investigate the deletion strain's growth on galactose, a spot assay was conducted. This procedure involved plating rows of the wildtype and deletion strains onto a YPD plate and a plate containing galactose. Each row of cells was 10 times more diluted than the next. The results of this assay again found that the deletion strain exhibited inhibited growth on galactose (**Fig.5)**.

Single Colony Purification

- After conducting the spot assay, single colony purification of the deletion strain on a plate containing both galactose and YPD was performed to account for the lack of growth medium in the previous assays. Again, the deletion did not grow.

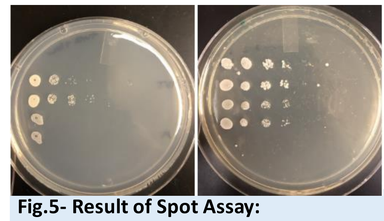


Fig.5- Result of Spot Assay: The tope 2 rows of colonies are the wildtype, while the bottom 2 rows are the deletion strain. Upon plating the deletion strain on galactose (left), no growth was observed compared to the growth on YPD (right).

The Possibility of a Petite Strain?

- The exhibited growth deficiency on galactose suggests that deletion strain is petite, which results from mitochondrial defects. Petite strains cannot survive on non-fermentable carbon sources and form significantly smaller colonies on fermentable carbon sources.[5]

Conclusions

- Data indicates that *Css3p* likely has a signal peptide and is non-cytoplasmic. Microscopy signaling screening suggests localization in the cell periphery. [2]
- As physical interactors with *Css3* have been shown to play a role in regulating mRNA decay and cytoskeletal processes, this raises the question of the role that *Css3* has in these processes. Another study has found that P-bodies are necessary for the posttranscriptional retrotransposition of Ty1 and suggests that P-bodies play a regulatory role in this process. [4] These interactions spark interest into the relationship that these elements have to one another.
- Further, the deletion strain's growth deficiency on galactose raise the question of whether this is a unique aspect of the deletion, or if the manufactured strain is petite.

Future Directions

- The deletion strain will be plated on glycerol to investigate whether it is petite. Deletion strains which are not petite will be used for future tests.
- Another transformation will be performed to incorporate a gene encoding green fluorescent protein (GFP) into the genome.
- *Css3p*-GFP fusion will be observed via fluorescent microscopy to better understand the protein product's physical location and interactions.

Sources

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2. https://www.yeastgenome.org/analyze
3. http://genemania.org/
4. Checkley, M. A., et al. "P-Body Components Are Required for Ty1 Retrotransposition during, Assembly of Retrotransposition-Competent Virus-Like Particles." *Molecular and Cellular Biology*, vol. 30, no. 2 Sept. 2009, pp. 382–398., doi:10.1128/mcb.00251-09.
5. https://en.wikipedia.org/wiki/Petite_mutation

Acknowledgements

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