Delving into the Function of YFR012W, a Regulator of Retro Transposition

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Abstract

This study aims to discover the function of YFR012W in *S. cerevisiae*. YFR012W, or DCV1, may have a role in the cell cycle. Additionally, deletion of DCV1 decreases activity of the Ty3 retroelement. Data from the Saccharomyces Gene Database reveals that the investigated gene has a paralogue, YOL019W, derived from a duplication in the genome. The information brought to light by bioinformatics modules reveals that both YOL019W and YFR012W are likely to code for integral membrane proteins. Currently, lab research is being done to investigate the effects of a dual knockout of both YOL019W and YFR012W. Both genes are viable when knocked out singularly, no information describes the effects of both genes being knocked out together. A yfr012w deletion mutant has been successfully created in a haploid strain with Kanmx and a deletion of YOL019W is underway. With the subsequent knockout of YOL019W, the haploid mutants yfr012w and yol019w will be mated and tetrad dissection will be performed, looking for two of four spores with a kanR gene. The transposition phenotype of the double deletion mutant will be tested. Bioinformatics has also revealed that, for yfr012w deletion mutants, there is a significant difference in bud growth direction and bud neck position in both bud size and nucleus location compared to wild type yeasts, this will be verified using microscopy.

Introduction

- *S. cerevisiae* is a single-celled eukaryote and is commonly known as baker's or brewer's yeast.[1]
- 10% of ORFs remain uncharacterized.
- YFR012W deletion mutation in the presence of 1-NM-PP1 shows a strong genetic interaction with cdc28-as1,[3] a key regulator of the cell cycle.[4] (Figure 1a)
- Deletion of YFR012W decreases activity of the yeast retrotransposon Ty3
- Ty3 transcription, translation and viral-like particle assembly relies on host genes of *S. cerevisiae*, in a way similar to mammalian retrovirus.[1] For transposition of Ty3, the RNA is packaged within viral-like particles where reverse-transcription will result in a full-length cDNA.[2] This cDNA is then integrated into the host gene by homologous recombination.[1] (Figure 2)
- YFR012W also has a paralogue, YOL019W, which arose from a whole genome duplication.[3] (Figure 1b)
- Paralogues are homologous genes whose function diverged within the species and of which both genes may now hold a related or a new function.

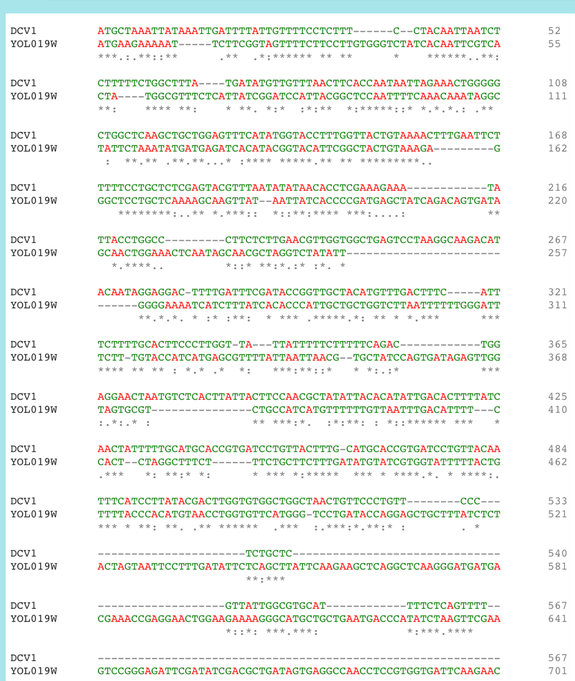

Figure 1. Using Clustal Omega, an alignment using the nucleic acid sequence of YFR012W and YOL019W, the gene's paralogue, was done. There is not a great amount of conservation between the paralogues. An asterisk under a sequence means that it is completely conserved, a colon means that there is conservation between amino acids with strongly similar properties, and a period means there is a conservation between amino acids with weakly similar properties. (https://www.ebi.ac.uk/Tools/msa/clustalo/)

Table 1. DCV1 is a part of the SUR7 protein domain family. A protein domain family is grouped based on tertiary protein structure rather than amino acid sequence. This table displays the genes apart of that family as well as their functions and locations. (https://www.yeastgenome.org/domain/PF06687)

Eisosomes: SUR7 forms a component of esiosomes, which were discovered in *S. cerevisiae* in 2006. They are large, polymorphic, immobile proteins at the plasma membrane involved in endocytosis.[5]

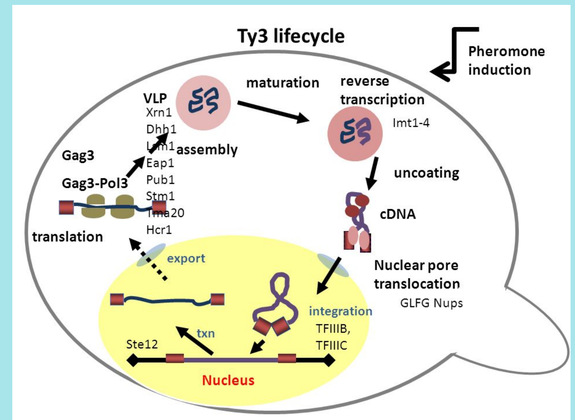

Figure 2. The lifecycle of Ty3 (https://slideplayer.com/slide/8063450/)

Bioinformatic Analysis

- Bioinformatic analysis using resources of the *Saccharomyces* Genome Database (yeastgenome.org) were done to investigate gene function.
- Modeling predicts that both YFR012W and the paralog YOL019W are transmembrane proteins. (Figure 3)
- YFR012W is also a part of the SUR7 protein domain family, all of the family members of which localize to the plasma membrane. Table 1 displays the members function and localization

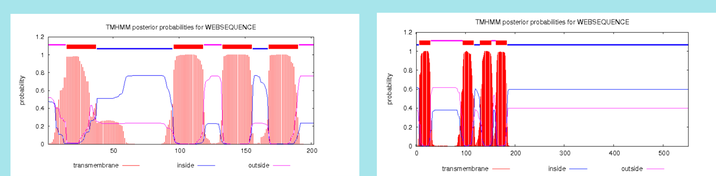

Figure 3. The Transmembrane Helices Hidden Markov Models. Modeling of YFR012W (left) and YOL019W (right) reveals that both proteins likely have four membrane domains, but with opposite polarity. The N and C-termini of YFR012W are predicted to have outward facing polarity (or facing into a vesicle), while the N and C-termini of YOL019W are predicted to have inward facing polarity. The cellular location of the proteins is not predicted by this algorithm. (http://www.cbs.dtu.dk/services/TMHMM/)

Gene	Description	Location
DCV1	Protein of unknown function; deletion mutant shows strong genetic interaction with cdc28-as1 mutant in the presence of 1-NM-PP1; DCV1 has a paralog,YOL019W, that arose from the whole genome duplication	Unknown
ECM7	Putative integral membrane protein with a role in calcium uptake; non-essential protein; mutant has cell wall defects and Ca+ uptake deficiencies; transcription is induced under conditions of zinc deficiency	Part of fungal type vacuole
FAT3	Protein required for fatty acid uptake; protein abundance increases in cortical patches in response to oleate exposure; the authentic, non-tagged protein is detected in a phosphorylated state in highly purified mitochondria in high-throughput studies; FAT3 has a paralog,YLR413W, that arose from the whole genome duplication	Mitochondria and cell periphery
FMP45	Integral membrane protein localized to mitochondria; required for sporulation and maintaining sphingolipid content; similar to SUR7; FMP45 has a paralog,YNL194C, that arose from the whole genome duplication	Cell cortex, cell periphery, mitochondria, plasma membrane
INA1	Protein of unknown function; not an essential gene;YLR413W has a paralog, FAT3, that arose from the whole genome duplication	Plasma membrane, cell periphery, fungal-type vacuole
PUN1	Plasma membrane protein with a role in cell wall integrity; co-localizes with Sur7p in punctate membrane patches; null mutant displays decreased thermotolerance; transcription induced upon cell wall damage and metal ion stress	Membrane rafts, plasma membrane, cell periphery, cellular bud, cytoplasm, endoplasmic reticulum
RIM9	Plasma membrane protein of unknown function; involved in the proteolytic activation of Rim101p in response to alkaline pH; interacts with Rim21p and Dfg16p to form a pH-sensing complex in the Rim101 pathway and is required to maintain Rim21p levels; has similarity to A. nidulans Pall	Plasma membrane protein
SUR7	Plasma membrane protein, component of eisosomes; long-lived protein that remains stable in eisosomes of mother cells while other eisosome proteins, Pil1p and Lsp1p, turn over; may function to anchor the eisosome in place; sporulation and plasma membrane sphingolipid content are altered in mutants; localizes to furrow-like invaginations (MCC patches)	Cell cortex, localizes with eisosome, membrane rafts, plasma membrane, cell periphery, mitochondrion
YNL194C	Integral membrane protein; required for sporulation and plasma membrane sphingolipid content; similar to SUR7; GFP-fusion protein is induced in response to the DNA-damaging agent MMS; GFP-fusion protein is more abundant at MCCs (membrane compartment occupied by Can1) in the presence of glycerol and oleate;YNL194C has a paralog, FMP45, that arose from the whole genome duplication	Cell cortex, cell periphery, cytoplasm, endoplasmic reticulum, plasma membrane
YOL019W	Protein of unknown function; green fluorescent protein (GFP)-fusion protein localizes to the cell periphery and vacuole;YOL019W has a paralog, DCV1, that arose from the whole genome duplication	Plasma membrane, cell periphery localization, part of fungal-type vacuole

Deletion Construction and Transposition assay

- A *YFR012W* deletion strain was created using homologous recombination of a PCR product containing the *kanmx* gene flanked by *YFR012W* upstream and downstream sequences. A transformation of the PCR into *S. cerevisiae* was followed by selection on G418 medium. Genomic DNA was isolated from transformants, and PCR using primers to the upstream region of *YFR012W* and *kanmx* confirmed deletion (figure 5).
- A Ty3 transposition assay utilizing a galactose inducible Ty3 element containing a histidine selectable marker confirmed that Ty3 transposition is decreased in a *YFR012W* deletion mutant

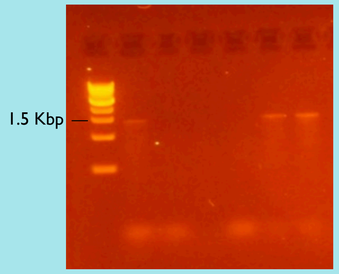

Figure 5. Gel electrophoresis to confirm the deletion of *YFR012W* using a forward primer upstream of the ORF and a reverse primer in *kanmx*. Three of six colonies are confirmed deletions, yielded the expected band of ~1.5 Kbp.

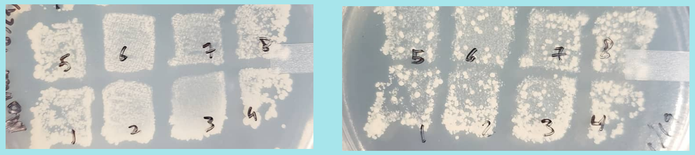

Figure 6. Ty3 transposition assay. Wild type (bottom row, patches 1-4) and *YFR012W* deletion (top row, patches 5-8) strains were transformed with a *URA3* selectable plasmid containing a galactose inducible Ty3 element and patched to glucose medium lacking uracil (left). The Ty3 element contains a *HIS3* marker gene with an artificial intron inserted. Splicing, reverse transcription, and integration of the Ty3 mRNA results in HIS+ prototrophs. Patches were printed to galactose medium lacking uracil (48 hours, 22°C) and then printed to glucose medium lacking histidine (right). Growth of papillae on media lacking histidine indicates transposition. The wild type strain appears to have slightly more transposition than the mutant strain.

Future Experiments

- A deletion mutant of *YOL019W* will be created. Both mutations have been found to be viable separately, yet the viability when deleted together is still to be investigated.
- The haploid deletion mutations of yol019w and yfr012w will be mated together and tetrad dissection looking for two of four spores with a *kanR* gene will be performed.
- Microscopy with calcofluor white stain of the haploid mutant yfr012w will be done to observe its phenotype in relation to bud neck growth direction and bud neck position.
- A DCV1, GFP tagged protein has been created and future investigation into protein localization via fluorescence microscopy is underway
- Within the genetic interactions of DCV1 is PHO5 and PHO23, both of which interact with repressible acid phosphatases. To explore this further, a *yfr012w* knockouts will be grown on phosphate limiting media

Acknowledgments

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