Investigating a Point Mutation in the *FUS1* Gene of *Saccharomyces cerevisiae*

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Background & Significance

Yeast have a very unique life cycle involving haploid cells that can fuse to create a diploid cell, then ultimately result in the production of spores to revert back to the haploid form (Figure 1).[2] Investigating the reproductive cycle in yeast is important as it enables us to understand the mechanisms of fusion in eukaryotic cells without controversially manipulating multicellular organisms. **Fusion is a very important cell function as it promotes important processes such as fertilization and organism growth, and the more we understand it and the methods involved, the higher the chance that we will be able to adequately address multicellular deformities and diseases resulting from irregular fusion.[1]**

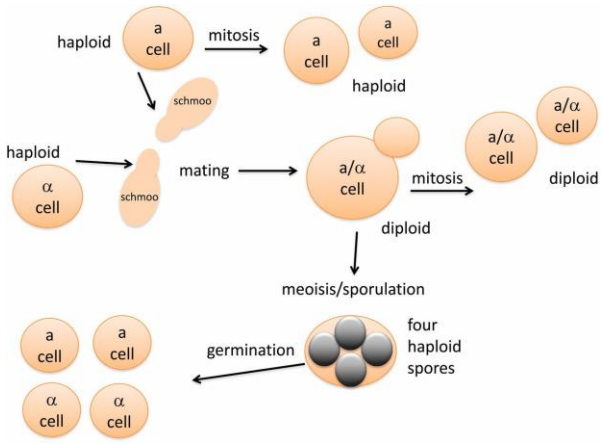

Figure 1: A representation of the reproductive cycle of yeast.[2] Two haploid cells can fuse together to form a diploid cell. Diploid cells can reproduce haploid cells by releasing spores. Haploid cells are either a or α, and two of the same type cannot fuse. Budding can occur as a diploid or haploid cell and is a form of asexual reproduction.

According to previous research on yeast cell fusion, the *FUS1* gene is an important player in the combination of two cells.[3–6] This gene is shown to be significant in cell wall degradation where the presence of fusion-promoting vesicles break down the cell wall to join two haploid cells into a diploid cell.[4,6] Correct positioning and release of these vesicles is very important as degradation of the cell wall too early, too late, or at an improper location could lead to failed fusion and potential cell death.[5] Most experiments done on the *FUS1* gene were knockout experiments concluding with results that showed that this gene had to be knocked out in all cells engaging in fusion in order to completely prevent fusion.[6] If only one of the cells involved in fusion had *FUS1* knocked out, then fusion would still occur as the vesicles from one cell would break down the wall of the other cell.[6] These results are very important because they lead to the understanding that *FUS1* not only plays an important role in the fusion of yeast cells, but that it most likely controls the movement, production, and release of the vesicles that physically allow fusion to occur.[4-6] However, there has been little research done on point mutations within the *FUS1* gene, and conducting experimental procedures changing specific residues produced in relation to this gene would lead to novel results. **Studying the results of point mutations is important because it will allow us to understand the specific residues that hold control in specific steps of cell fusion, and how altering these residues will ultimately impact the ability of the cell to undergo fusion.**

Approach

Experimental Approach: We hypothesize that the point mutation made in the *FUS1* gene will completely block the fusion of the two cells. This prevention could occur by altering the function of the fusion-promoting vesicles that degrade the cell walls of the yeast and allow assimilation of cell contents. We assume this would happen because we will be performing the residue mutation F262A by making the base mutation TT784GC. These two amino acids are drastically different from one another, as phenylalanine is very hydrophobic with an aromatic group, and alanine is a much smaller molecule with a terminal CH_3 group. These differences in amino acid properties will most likely alter how the translated protein behaves and interacts with other surrounding molecules and residues.

To make this point mutation, we will use site-directed mutagenesis, a method of creating intentional mutations at a specific location in the genome. We will perform a polymerase chain reaction (PCR) of the mutated *fus1* gene using the FUS1-GFP plasmid isolated from bacteria. We will create three different transformation groups with *S. cerevisiae.* The first group will be transformed with the mutant plasmid, the second group will be transformed with the wildtype plasmid, and the third group will be transformed with an empty vector. The empty vector allows for an analysis to determine if the transformation process itself had any cytotoxic effects on the cell before concluding that those cellular changes occurred as a consequence of the mutated DNA. We will allow the transformants to sufficiently multiply then we will observe fusion phenotypes using a plate mating assay. This plating method is highly organized and allows for the identical location transfer of the mutants to several plates

for further analyzation. Mating success will be determined by transferring mutants grown on a nutrient rich media onto a selective media for diploids. If mating is successful, then a group will experience a large amount of growth on the diploid selective plate.

Possible Outcomes: There are three possible outcomes that could result from this experiment: 1) The point mutation will completely block the fusion of the two yeast cells, 2) the point mutation will partially block the fusion of the two yeast cells, potentially preventing some cell wall degradation or complete fusion of the two cells into one, or 3) the point mutation will have no effect on the ability of the two cells to fuse (Figure 2).[5] If the results show potential outcome 1 and fusion is completely blocked, then we will more closely analyze the differences in the mechanism between the mutant and the wildtype. We will do this by using an electron microscope and observing the movement of the different vesicles at the different stages of cell fusion. If the results show potential outcome 2 and fusion is partially blocked, then we will also analyze the movement of different vesicles at the different stages of fusion, but we will also consider the possibility of multiple residues produced by the *FUS1* gene possessing control over the aspects of cell fusion. We would perform another point mutation in the *FUS1* gene while maintaining this mutation in order to determine if several point mutations are necessary to interrupt the process of cell fusion. If the results show potential outcome 3 and fusion ability is not altered, then we will perform another point mutation to the 262nd phenylalanine in the *FUS1* gene by changing it to an arginine, as this amino acid has a very long carbon chain with charged NH_2 groups on the end. The alanine may not produce a drastic enough change in protein structure since phenylalanine is a derivative of alanine.

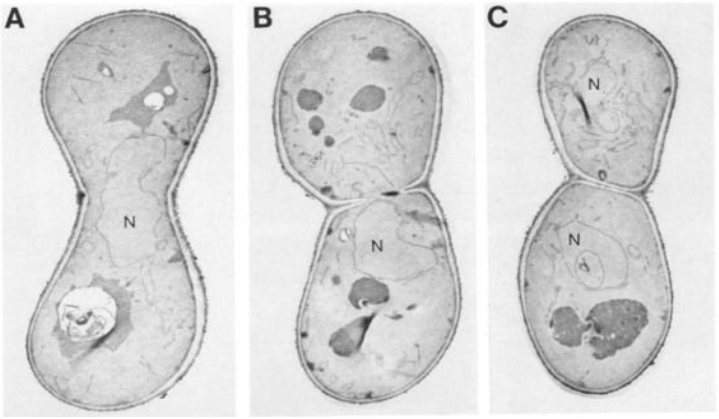

Figure 2: A representation of the three possible outcomes from this experiment.[5] A) The point mutation has no effect on the ability of the cells to fuse. B) The point mutation prevents partial degradation of the cell walls and the cells partially fuse. C) The point mutation completely prevents the two cells from fusing.

Summary

I will determine how a point mutation in the *FUS1* gene of the yeast *Saccharomyces cerevisiae* affects the ability of two haploid cells to fuse into one diploid cell. The comparison of wildtypes to mutants in regards to the fusion phenotype will provide insight on the mechanisms allowing an important cellular function to occur.

References

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