Role of a transport protein in localizing *ASH1* mRNA in a harmful yeast

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Candida albicans is a fungus that causes minor infections in healthy individuals, but can result in fatal bloodstream infections among people with weakened immune systems (Inglis & Johnson, 2002). *Candida* can exist in distinct forms. As a budding yeast, it takes the form of single round/oval-shaped cells. The alternative hyphal form consists of long tube-like cells. The ability of *C. albicans* to switch between these forms is essential as it allows it to efficiently respond to environmental changes. When *C. albicans* switches from a budding yeast to a hypha, its ability to infect other organisms becomes enhanced. It is imperative to try to understand the characteristics that underlie the infectiousness of *C. albicans*, considering the health problems that arise from its successful infection of a host.

The yeast-to-hyphal switch requires a protein called Ash1 (Inglis & Johnson, 2002). The instructions to build proteins are called genes, and are found in the DNA that is responsible for making the protein. For the protein-building cellular machinery to understand these instructions, they need to be re-written in a form known as messenger RNA (mRNA). After the *ASH1* mRNA is made, it is carried over to the hyphal tip where the machinery responsible for making Ash1 reads the mRNA and makes the protein. The Ash1 protein makes the cell produce other proteins which help it switch to a hyphal form (Inglis & Johnson, 2002), or pseudohyphal form in the case of *S. cerevisiae* (Ryan et al., 2012). Although it is known that a protein called She3 plays a role in carrying the *ASH1* mRNA to the cell tip, its function in this process is not fully understood in *C. albicans* (Elson et al., 2009).

ASH1 mRNA transport is well understood in baker's yeast (*Saccharomyces cerevisiae*), which shares some similarities with *C. albicans* (Landers et al., 2009). Since both species use their respective versions of She3 to transport *ASH1* mRNA to the tip of hyphal or pseudohyphal cells, we intend to use *S. cerevisiae* — which does not cause infectious disease — as a tool to try to better understand the full function of the *C. albicans* She3 in the transport process. In *Saccharomyces cerevisiae, ASH1* mRNA is transported by a complex consisting of the She3, She2, and Myo4 proteins (Shi et al., 2014). A significant difference between the *ASH1* mRNA transport of the *C. albicans* and *S. cerevisiae* is that the latter requires both the She2 and She3 proteins, whereas the former does not possess the She2 protein. (Elson et al., 2009). Another point of difference is in the motor proteins present in the two species of yeast (Elson et al., 2009). How then does *ASH1* mRNA transport occur in *C. albicans*? One possibility is that the *Candida* She3 is able to — by itself — fulfill the roles observed in both She3 and She2 in *S. cerevisiae*. An alternative possibility is that there may be one or more proteins in *C. albicans* that enable the successful transport of *ASH1* mRNA in the absence of She2. Additionally, *Ashbya gossypii* is a filamentous fungus that is found between *S. cerevisiae* and *C. albicans* in the evolutionary tree. An interesting feature about *Ashbya* is that it possesses both She2 and She3, which it might use for *ASH1* mRNA transport. The goal of my project is to test whether the She3 of *A. gossypii* and/or *C. albicans* can successfully transport *ASH1* mRNA in *S. cerevisiae*.

Over the eight weeks of this summer fellowship, I have extensively delved into literature relevant to *ASH1* mRNA transport in *Saccharomyces cerevisiae*. From this literature, I have designed an experiment to assess the functionality of *C. albicans* or *A. gossypii* She3 in *Saccharomyces cerevisiae*. My experiment will involve copying the *SHE3* genes of *C. albicans* and *A. gossypii* by a process called Polymerase Chain Reaction (PCR), and incorporating these genes into *S. cerevisiae*. Yeast, like bacteria, possess small circular DNA molecules called plasmids. I will insert a copy of the *C. albicans* and *A. gossypii SHE3* gene into a plasmid, and put these plasmids into *S. cerevisiae* cells that are unable to make their own She3 proteins. These cells will be grown in conditions that require She3 for growth, and observed under a microscope for the formation of pseudohyphae; *S. cerevisiae* cells that have no She3 have a substantially reduced ability to form pseudohyphae (Ryan et al., 2012). The strain for my proposed growth assay can be obtained from Ryan et al., 2012. A vacant plasmid from Robert Sikorski's collection can be used produce *Candida* She3 in *Saccharomyces* cells. Based on the greater similarity between the She3 of *A. gossypii* and *S. cerevisiae*, I propose that the *A. gossypii* She3 is more likely than the *C. albicans* She3 to successfully transport *ASH1* mRNA to the cell tip in *S. cerevisiae*, and will hence display greater or more efficient pseudohyphal formation.

This summer research fellowship was originally intended to involve in-lab experiments that would be carried out on-campus. Due to the global pandemic that has taken the world by storm, the intended plan could not be implemented. However, the remote research alternative that I have pursued has certainly been worthwhile in terms of intellectual engagement and growth, as well as setting me up well for my intended honors project in the fall. I would like to thank the Student Fellowships Committee for giving me the opportunity to engage in this lucrative summer project. I would also like to extend my gratitude to the donors behind the Life Sciences Fellowship that has funded my work.

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