Testing the Importance of a Possible mRNA Transport Protein for Hyphal Growth in the Pathogenic Fungus *Candida albicans*

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Candida albicans is a common fungus that lives in humans and can lead to disease in people with compromised immune systems. C. albicans has two major forms known as spherical yeast cells and elongated filamentous hyphal cells (Sudbury 2011). The yeast form allows C. albicans to travel in the hosts' bloodstream. The hyphal form expresses protein at the hyphal tip that aids in host cell attachment and host-tissue invasion (Sudbury 2011). In the hyphal form, proteins are concentrated at the hyphal tip through mRNA transport. She3 is the major transport protein acting in this system. She3 moves messenger RNA from the mother cell to an extended and invasive "hypha" (Elson et al, 2009). Messenger RNA (mRNA) carries a copy of the genetic information for a specific protein of interest. This She3 complex is then transported to the hyphal tip to begin the production of select proteins important for hyphal function and virulence (Elson et al, 2009). By understanding the mRNA transport system, including other proteins that work with She3, we can understand how these proteins contribute to the formation of *C.albicans* invasive hyphal tip.

A study showed the deletion of She3 led to hyphal defects and reduction in invasive growth which suggests that the She3-mediated transport system impacts the filamentation of *C.albicans* (Elson et al, 2009). Previously, the McBride lab purified SHE3 and identified proteins bound that copurified with SHE3 (Pholcharee et al., 2018) One of the interacting proteins of SHE3 identified by copurification was IPS2, a known mRNA-binding protein that is found in other closely related fungal species. This suggests that She3 is aided by other mRNA-binding proteins to effectively transport the mRNA to the hyphal tip for protein expression.

This summer I aimed to understand how the deletion of the *Ips2* gene would impact hyphal growth in Candida albicans. Since IPS2 was found to interact with SHE3, I hypothesized that the deletion of IPS2 genes would produce filamentation defects in C. albicans that are similar to the filamentous defects found in the absence of *She3* genes. To approach the experiment, I deleted both copies of the IPS2 gene and then observed hyphal formation on solid and liquid medium.

Arginine (Arg) and histidine (His) are two amino acids that are necessary for cellular growth. Using *C. albicans* cells that require Arg and His, I replaced the *Ips2* genes with genes that encoded for enzymes that will allow the cells to make these amino acids. Then I selected for successful replacement by growing the cells on solid media that did not contain arginine and/or histidine. Since cells have the gene, they should be able to produce arginine and histidine to survive. The deletion of *Ips2* from the genome proved to be difficult as both copies of IPS2 were successfully deleted in only five percent of colonies.

Then I grew the newly constructed cells without *Ips2* on hyphal inducing and non-inducing plates to observe filamentation. I compared its growth to the wildtype strain on the same plates. On non-hyphal inducing plates, the absence of IPS2 leads to slower growth since the spot was smaller in size. Unlike the wildtype strain on filament-inducing plates, the *Ips2* double deletion strain lacked a central wrinkled region. The mutant strain displayed little to no sign of invasive growth as indicated by the lack of a peripheral region or "halo" surrounding the original spot. The *Ips2* double deletion strain also appeared to be more translucent in comparison. This agrees with my hypothesis that IPS2 participates in the She3 mRNA transport system. Since *Ips2* and *She3* mutant strains do not display the same defects it suggests that IPS2 may participate in other cellular processes than only the mRNA transport system. However, in a hyphal inducing liquid media, there was no significant difference between the lengths of hyphae found in IPS2 deletion and wildtype strain. There was no difference between IPS2 and SHE3 deletion strains.

Moving forward, future research should aim to uncover the specific function of IPS2 in the She3 transport system. This could involve determining if specific mRNA bind to IPS2 and what they encode for, the localization of the mRNA transcripts in cells without IPS2, and if the deletion of *Ips2* impacts virulence and invasive growth in C. albicans.

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References

Elson SL, Noble SM, Solis NV, Filler SG, Johnson AD. 2009. An RNA transport system in Candida albicans regulates hyphal morphology and invasive growth. PLoS Genet 5(9):e1000664.

Pholcharee T. 2018. Exploring mechanisms of mRNA localization through the identification of RNA-binding protein complexes in the pathogenic fungus Candida albicans.

Sudbery PE. 2011. Growth of Candida albicans hyphae. Nat Rev Microbiol 9(10):737-748.