Investigating the impact of an RNA-binding protein on hyphal tip localization of an mRNA in *Candida albicans*

Alaijah Rubianes, Class of 2024

Candida albicans is an opportunistic pathogenic fungus that can enter the bloodstream & internal organs, which can lead to life-threatening infections for immunocompromised people [1, 2]. Infection rate is linked to the transformation of budding yeast cells to hyphal cells [3]. Hyphal cells express different proteins at the hyphal tip including adhesions and digestive enzymes to damage host tissue. In cells, gene expression is the process of stored information in DNA being used as a template to make "messenger" RNA (mRNA), which creates proteins important for cell function. Gene expression impacts where the proteins, and the mRNAs that encode the proteins, are, which ultimately can have a significant impact on cell growth and function. As a result, it is important to understand how exactly gene expression impacts hyphal formation. For appropriate hyphal development and function, some mRNA needs to be transported to the hyphal tip [4].

One of the most studied models of fungal mRNA localization is the baker's yeast "She complex" [5,6]. The major mRNA binding protein inside the She complex, She2, links certain mRNAs to a motor protein, through the adaptor protein She3. This results in the mRNAs being transported from the mother cell to the daughter cell, where proteins can then be synthesized [5,6].

Although *C. albicans* contains She3 and motor proteins, one distinguishing aspect between baker's yeast and *C. albicans* is that the latter fungus does not contain She2 [4]. Therefore, some other mechanism must be used to take transported mRNAs to She3, which is important for the normal hyphal formation and transporting some mRNAs to the hyphal tip [4]. One protein that may replace the function of She2 is Slr1, an RNA-binding protein that affects hyphal growth [7]. *ASH1* is a specific mRNA that is transported by She3 in both baker's yeast and *C. albicans* [5,6] This summer, the purpose of my research project was to test whether the Slr1 protein helps transport *ASH1* mRNA to the hyphal tip. To test this hypothesis, I collaborated with Ali Hussein to determine and quantify where *ASH1* mRNA is found in cells with and without Slr1.

To visualize the location of *ASH1* mRNA in *C. albicans* cells, we used a technique called fluorescent in situ hybridization (FISH). A fluorescent dye is linked to probes that will only bind to *ASH1* mRNA. This multi-step process involved the growth of hyphal cells followed by treatment to allow the probes to enter the cell. By looking at the cells under a confocal microscope, we quantified the number of cells with *ASH1* mRNA at the hyphal tip[4]. To analyze the images, we compared normal mRNA localization at the hyphal tip in positive "wildtype" (WT) control cells to mRNA localization in Slr1 deleted cells. Using the same FISH procedure in the last week of experiments, we tested *ASH1* localization in cells without a protein called Ips1, a protein that copurified with She3 in previous experiments in the McBride lab.

Initial results indicate that Slr1 is not essential for mRNA transport. Additionally, increasing the time of hyphal formation from 60 to 80 minutes increases *ASH1* mRNA localization to the hyphal tip in both WT and Slr1 deleted cells. Compared to WT and Slr1-deleted cells, Ips1 deleted cells showed a lower percentage of hyphae with *ASH1* mRNAs at the tip. This result suggests that this protein may have a more direct involvement on mRNA transport, but more data analysis is needed. These findings contribute to the knowledge of the diversity in gene expression mechanisms between different species of fungus.

Faculty Mentor: McBride
Funded by the Kibbe Science Fellowship
References:
1. Candida infections of the mouth, throat, and esophagus | Fungal Diseases | CDC. 2021 Feb 25. https://www.cdc.gov/fungal/diseases/candidiasis/thrush/index.html
2. Buchacz K, Lau B, Jing Y, Bosch R, Abraham AG, Gill MJ, Silverberg MJ, Goedert JJ, Sterling TR, Althoff KN, et al. Incidence of AIDS-Defining Opportunistic Infections in a Multicohort Analysis of HIV-infected Persons in the United States and Canada, 2000–2010. The Journal of Infectious Diseases. 2016;214(6):862–872. doi:10.1093/infdis/jiw085
3. Lo HJ, Köhler JR, DiDomenico B, Loebenberg D, Cacciapuoti A, Fink GR. Nonfilamentous C. albicans mutants are avirulent. Cell. 1997;90(5):939–949. doi:10.1016/s0092-8674(00)80358-x
4. Elson SL, Noble SM, Solis NV, Filler SG, Johnson AD. An RNA transport system in Candida albicans regulates hyphal morphology and invasive growth. PLoS genetics. 2009;5(9):e1000664. doi:10.1371/journal.pgen.1000664
6. Shepard KA, Gerber AP, Jambhekar A, Takizawa PA, Brown PO, Herschlag D, DeRisi JL, Vale RD. Widespread cytoplasmic mRNA transport in yeast: Identification of 22 bud-localized transcripts using DNA microarray analysis. Proceedings of the National Academy of Sciences of the United States of America. 2003;100(20):11429–11434. doi:10.1073/pnas.2033246100
7. Ariyachet C, Solis NV, Liu Y, Prasadarao NV, Filler SG, McBride AE. SR-like RNA-binding protein Slr1 affects Candida albicans filamentation and virulence. Infect Immun. 2013 Apr;81(4):1267-76. doi: 10.1128/IAI.00864-12. Epub 2013 Feb 4. PMID: 23381995; PMCID: PMC3639594.