The effect of mRNA binding protein Slr1 on transport of mRNAs in *Candida albicans*

Zoe Dietrich, Class of 2021

The yeast *Candida albicans* is a common human fungal pathogen that can cause diseases ranging in severity from minor infection of the skin to the fourth most common hospital-acquired bloodstream infection in the United States, with a mortality rate of up to 71 percent [1]. Because yeast species have exhibited increasing resistance to antifungal drugs [2], a deeper understanding of the cellular mechanisms that allow yeast cells to invade host cells must be developed.

To infect animal models, yeast cells must be able to transition between a circular shape and a hyphal or elongated shape [3,4]. Different proteins expressed in the hyphal form but not in the yeast form allow the hyphal form to infect host cells. One mechanism that another fungus uses to differentially express proteins and transition between shapes is called directional mRNA transport [5]. mRNAs are fragments of genetic material that are used to make proteins within cells. Forming certain proteins in specific regions of cells is one of the mechanisms that drives cells to change shape. Transporting mRNA to specific regions allows formation of proteins at their sites of function, instead of near the nucleus, the structure containing their genetic material [6]. Directional mRNA transport could be one of the mechanisms by which *C. albicans* cells transition between circular and elongated hyphal shapes and differentially express proteins that allow for stronger host cell adherence of the hyphal form. Evidence for this hypothesis involves She3, an RNA-binding protein that transports mRNA from the nucleus to a region of the yeast cell important for hyphal formation known as the hyphal tip. When She3 is missing from the cell, the hyphal cell cannot form properly, suggesting that directional mRNA transport is necessary for hyphal function [7]. As only two studies have been published on mechanisms of mRNA transport in *C. albicans* [7,8], further research concerning these mechanisms and their role in host cell invasion is necessary.

The overall goal of the McBride lab is to understand mRNA transport in hyphal cells and the role it plays in hyphal growth and function. We are interested in a protein known as Slr1, which is similar to She3 in that it also binds mRNA. *C. albicans* cells without Slr1 exhibit decreased hyphal growth and a diminished ability to damage host cells [9]. Localization of a form of Slr1 to the hyphal tip is partially dependent on the presence of She3, suggesting that Slr1 may act as an mRNA transport protein associated with She3 complexes in *C. albicans* hyphae [8]. Our research aims to determine if Slr1 is a functional component of the She3 mRNA transport complex in *C. albicans* hyphal cells.

My specific goal this summer was to understand if transport of the *ASH1* mRNA by She3 complexes to the hyphal tip is affected by the presence of Slr1. To test for this relationship, I collaborated with Aimee An to develop a technique known as fluorescence *in situ* hybridization, or FISH, allowing us to visualize where *ASH1* mRNA was located in *C. albicans* cells. Fluorescently tagged fragments of DNA that could bind to the *ASH1* mRNA, known as probes, allowed visualization of the transported mRNA. To insert the probes into the hyphal cells, we digested the cell walls and permeabilized the cell membranes; next, we visualized the probes with microscopy and digital imaging to identify the location of *ASH1* mRNA in the cells.

We used this technique in cells with Slr1 and She3, cells without Slr1, and cells without She3. Cells without She3 served as a control, as transported mRNAs should not localize to the hyphal tip in the absence of She3 [4]. If Slr1 is a functional component of the She3 mRNA transport complex, we would expect to see more *ASH1* mRNA in the hyphal tip in the presence compared to the absence of Slr1. After optimization of the FISH technique, an initial blind experiment showed localization of *ASH1* mRNA to the hyphal tip in cells with both Slr1 and She3, but no specific localization in cells without Slr1, indicating that Slr1 could work with She3 to transport these mRNAs to the hyphal tip. More experiments are needed to further optimize FISH and confirm this result.

Faculty Mentor: Anne McBride

Funded by INBRE, IDeA Networks of Biomedical Research Excellence, and NIH

References Cited

[1] Falagas M, Apostolou K, Pappas V. 2006. Attributable mortality of candidemia: a systematic review of matched cohort and case-control studies. Eur J Clin Microbiol Infect Dis. 25: 419-425.

[2] Odds F, Brown A, Gow N. 2003. Antifungal agents: mechanisms of action. Trends Microbiol. 11: 272-279.

[3] Lo H, Kohler J, DiDomenico B, Loebenberg D, Cacciapuoti A, Fink G. 1997. Nonfilamentous *C. albicans* mutants are avirulent. Cell. 90: 939-949.

[4] Saville S, Lazzell A, Monteagudo C, Lopez-Ribot J. 2003. Engineered control of cell morphology in vivo reveals distinct roles for yeast and filamentous forms of *Candida albicans* during infection. Eukaryot Cell. 2: 1053-1060.

[5] Becht P, König J, Feldbrügge M. 2006. The RNA-binding protein Rrm4 is essential for polarity in *Ustilago maydis* and shuttles along microtubules. Journal of Cell Science. 119: 4964-4973.

[6] Jung H, Gkogkas C, Sonenberg N, Holt C. 2014. Remote control of gene function by local translation. Cell. 157: 26-40.

[7] Elson S, Noble S, Solis N, Filler S, Johnson A. 2009. An RNA transport system in *Candida albicans* regulates hyphal morphology and invasive growth. PLoS Genet. 5: e1000664.

[8] Ariyachet C, Beißel C, Li X, Lorrey S, Mackenzie O, Martin PM, O'Brien K, Pholcharee T, Sim S, Krebber H, McBride AE. 2017. Post-translational modification directs nuclear and hyphal tip localization of *Candida albicans* mRNA-binding protein Slr1. Mol Microbiol. 104(3):499-519.

[9] Ariyachet C, Solis N, Liu Y, Prasadarao N, Filler S, McBride A. 2013. SR-like RNA-binding protein Slr1 affects *Candida albicans* filamentation and virulence. Infect Immun. 81: 1267-1276.