**Investigating the function of SR-like RNA-binding protein (Slr1) in fungal RNA transport**

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*Candida albicans* (*C. albicans*) is a fungus commonly found on human skin and genitalia or in the mouth and gut. *C. albicans* can cause a range of diseases in immunocompromised hosts including candidiasis [5,11]. The virulence of *C. albicans* is related to changes in its form where the cell switches from its normal, circular shape to an elongated form known as a hypha. Hypha is associated with sticking to and invading host cells: both of which are crucial steps for the early stages of infection [4,10,11]. Genetic material known as *ASH1* mRNA is required for hyphal formation and is transported to the hyphal tip by transport proteins [4]. Once at the tip, *ASH1* mRNA is translated from the mRNA into Ash1 proteins which have essential functions to allow hyphal growth. While hyphal growth has been well-studied, there are still many questions about the *C. albicans ASH1* transport mechanism. Understanding hyphal formation can help us better understand the *C. albicans* infection model.

The *ASH1* mRNA transport mechanism is well-studied in the related budding yeast species. In budding yeast, *ASH1* mRNA is asymmetrically transported by She2 and She3 proteins [7-9]. In *C. albicans*, only She3p is found [3]. In the absence of She3p, there are defects in hyphal growth suggesting that She3p transport of mRNAs such as ASH1 plays a role in hyphal growth [3]. Does *C. albicans* use other proteins for transport and does *C. albicans* have a similar protein like She2p? The SR-like RNA-binding protein Slr1 is a protein candidate that may be involved with *ASH1* transport [1]. Slr1 can bind to *ASH1* mRNA and impacts filamentation and growth [2, 13, 14].

This summer, my goal was to investigate whether Slr1 had a role in the *C. albicans* She3-mediated transport system. To test this, I conducted fluorescent in situ hybridization (FISH) experiments, a technique that can detect and locate *ASH1* mRNAs in cells with or without various proteins. The FISH technique allows the addition of a fluorescent tag to *ASH1* mRNA which can be observed as a red dot with a confocal microscope. I captured images and quantified the cells based on where the *ASH1* mRNA was seen within each cell. *ASH1* mRNA only localizes to the tip in cells that have already copied and divided their DNA into two nuclei ("post-mitotic cells") [3,12]; therefore, I only quantified post-mitotic cells showing red dots indicating *ASH1* mRNA (Figure 1). As expected, wildtype (WT) cells showed the most frequent tip localization of *ASH1* mRNA (63%) while none of the cells without She3 showed tip localization (0%). Interestingly, 26% of cells without Slr1 had *ASH1* mRNA localized at the hyphal tip.

My results suggest that Slr1 is not required for *ASH1* transport, but Slr1 may indirectly impact *ASH1* localization. The absence of Slr1 reduced but did not completely impede tip localization suggesting that the absence of Slr1 does not have as much of an impact on *ASH1* localization as the absence of She3 (a known *ASH1* transport protein). Potentially, Slr1 might be involved in mRNA splicing, chromatin modification, and mRNA nuclear export which might impact where *ASH1* is found in the cell [14]. To understand the *ASH1* transport system better, future studies will focus on testing other proteins candidates for direct roles in cytoplasmic transport of *ASH1* mRNA.

This project has allowed me to practice important lab skills such as pipetting, light microscopy, confocal microscopy, aseptic techniques, and hazardous waste management. I will continue to apply these skills in my honors project and in post-undergraduate lab positions.

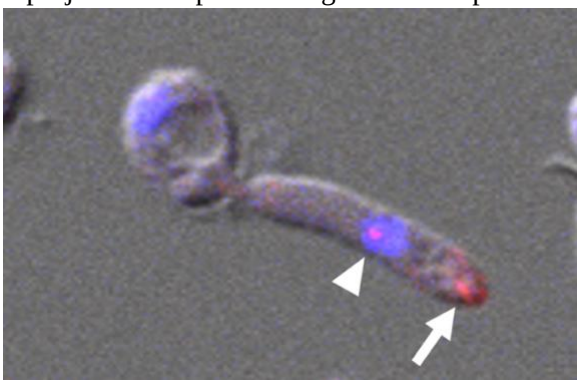

Figure 1. FISH experiment images captured on the confocal microscope. Localization was observed as a bright red spot. Here, the WT cell showed tip localization (arrow) and non-tip localization (arrowhead). The red spots indicate *ASH1* mRNAs. The blue spots are the two nuclei.

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## References


1 Ariyachet, C. *et al.* Post-translational modification directs nuclear and hyphal tip localization of *Candida albicans* mRNA-binding protein Slr1. *Mol Microbiol* **104**, 499-519, doi:10.1111/mmi.13643 (2017).

2 Ariyachet, C. *et al.* SR-like RNA-binding protein Slr1 affects *Candida albicans* filamentation and virulence. *Infect Immun* **81**, 1267-1276, doi:10.1128/IAI.00864-12 (2013).

3 Elson, S. L., Noble, S. M., Solis, N. V., Filler, S. G. & Johnson, A. D. An RNA transport system in *Candida albicans* regulates hyphal morphology and invasive growth. *PLoS Genet* **5**, e1000664, doi:10.1371/journal.pgen.1000664 (2009).

4 Inglis, D. O. & Johnson, A. D. Ash1 protein, an asymmetrically localized transcriptional regulator, controls filamentous growth and virulence of *Candida albicans*. *Mol Cell Biol* **22**, 8669-8680, doi:10.1128/mcb.22.24.8669-8680.2002 (2002).

5 Kadosh, D. Control of *Candida albicans* morphology and pathogenicity by post-transcriptional mechanisms. *Cell Mol Life Sci* **73**, 4265-4278, doi:10.1007/s00018-016-2294-y (2016).

6 Lange, S. *et al.* Simultaneous transport of different localized mRNA species revealed by live-cell imaging. *Traffic* **9**, 1256-1267, doi:10.1111/j.1600-0854.2008.00763.x (2008).

7 Long, R. M., Gu, W., Lorimer, E., Singer, R. H. & Chartrand, P. She2p is a novel RNA-binding protein that recruits the Myo4p-She3p complex to *ASH1* mRNA. *EMBO J* **19**, 6592-6601, doi:10.1093/emboj/19.23.6592 (2000).

8 Muller, M. *et al.* A cytoplasmic complex mediates specific mRNA recognition and localization in yeast. *PLoS Biol* **9**, e1000611, doi:10.1371/journal.pbio.1000611 (2011).

9 Takizawa, P. A. & Vale, R. D. The myosin motor, Myo4p, binds *Ash1* mRNA via the adapter protein, She3p. *Proc Natl Acad Sci U S A* **97**, 5273-5278, doi:10.1073/pnas.080585897 (2000).

10 Verma-Gaur, J. & Traven, A. Post-transcriptional gene regulation in the biology and virulence of *Candida albicans*. *Cell Microbiol* **18**, 800-806, doi:10.1111/cmi.12593 (2016).

11 Zdanaviciene, E., Sakalauskiene, J., Gleiznys, A., Gleiznys, D. & Zilinskas, J. Host responses to Candida albicans. A review. *Stomatologija* **19**, 109-123 (2017).

12 Beane, E. *Role of SR-like RNA-binding protein 1 (Slr1) in hyphal tip localization of She3-transported mRNA in Candida albicans*, Bowdoin College, (2020).

13 Landes, E. "*Investigating binding specificity of RNA-binding proteins in the pathogenic fungus Candida albicans*, Bowdoin College, (2019).

14 Pholcharee, T. *Exploring mechanisms of mRNA localization through the identification of RNA-binding protein complexes in the pathogenic fungus Candida albicans*, Bowdoin College, (2018).