

## 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<sup>5,11</sup>. 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<sup>4,10,11</sup>. Genetic material known as *ASH1* mRNA is required for hyphal formation and is transported to the hyphal tip by transport proteins<sup>4</sup>. 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<sup>7-9</sup>. In *C. albicans*, only She3p is found<sup>3</sup>. 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<sup>3</sup>. 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<sup>1</sup>. Slr1 can bind to *ASH1* mRNA and impacts filamentation and growth<sup>2,13,14</sup>.

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 post-mitotic cells. In WT cells, 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<sup>14</sup>. 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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