Pathogenic growth and mRNA localization in pathogenic fungus *Candida albicans*

Emma Beane, Class of 2020

Candida albicans, an opportunistic fungal pathogen, can cause a wide variety of infections in humans, including more widespread infections in immunocompromised patients (Noble and Johnson, 2007). *C. albicans* can transition from a budding, circular form to an elongated hyphal form, which aids in host cell adhesion and invasion during infection (Elson et al., 2009). Certain proteins are found specifically at the elongated end, or tip, of hyphal cells which assist in these processes. The question is how do these proteins get to the tip? There are two potential mechanisms: protein transport and mRNA transport. In mRNA transport, mRNA, the DNA code for protein, is carried from the nucleus, which houses the DNA, to the hyphal tip by a transport protein where it is translated on site into protein. A transport protein, called She3, has been identified in *C. albicans* and is known to transport a subset of mRNAs to the hyphal tip (Elson et al., 2009). There may be an RNA-binding protein that carries the mRNA from the nucleus into the cytoplasm of the cell where She3 can be found; however, which protein that is remains unknown. One potential candidate for this RNA-binding protein is called Slr1. Slr1 is involved in cell growth and hyphal formation (Ariyachet et al., 2013), and associates with a fraction of the mRNAs transported by She3 (McBride lab, unpublished data). We wanted to investigate whether Slr1 was a functional component of the She3 mRNA transport system, and more specifically understand how Slr1 impacts the transport of She3-transported mRNAs to the hyphal tip.

To investigate this question, we used an approach referred to as fluorescent *in situ* hybridization (FISH) to visualize the location of mRNA in hyphal cells. FISH utilizes "probes" that recognize and bind to a specific mRNA. Probes are fluorescently tagged and can be identified using microscopic imaging allowing us to visualize the location of *ASH1* mRNA, a known She3-transported mRNA. In our experiment, we tested four different versions of cells. The first version was wildtype (WT), in which we expected to see *ASH1* mRNAs localized at the hyphal tip. The second version did not have the She3 protein ("she3 deleted"), and we expected to see *ASH1* mRNA diffuse through the hyphal cells rather than localized at the tip (Elson et al., 2009). The third version did not have the Slr1 protein ("slr1 deleted"), and we expected to see similar results to she3 deleted cells if Slr1 plays a key role in the She3 transport system. The fourth version did not have the gene that codes for *ASH1* mRNA ("ash1 deleted"), so we did not expect to see *ASH1* mRNA present. This fourth version of cells served as a negative control, meaning we could tell if probes were binding non-specifically within the cells. For WT and ash1 deleted cells, our expected results were supported. Approximately 16 percent of WT hyphae showed localization at the tip. For she3 deleted cells, we saw *ASH1* mRNA diffused throughout the cell with some localized around the nucleus; however, for slr1 deleted cells, we did not see any signal that would indicate *ASH1* was present. We wanted to ensure that this lack of signal was due to a lack of localization rather than a lack of *ASH1* mRNA present in the slr1 deleted cells.

To investigate mRNA expression levels, we used reverse transcription quantitative polymerase chain reaction (RT-qPCR) which allows us to compare relative quantities of *ASH1* mRNA to WT. *ASH1* mRNA levels were not strongly reduced in either the she3 deleted or the slr1 deleted cells as compared to WT, which led us to conclude that the lack of signal in our FISH experiment was due to a lack of localization as supposed to reduced mRNA levels. Based upon these experiments, it appears that the absence of Slr1 protein results in no hyphal tip localization; however, our most recent experiment yielded a small percentage (about 1.5 percent) of hyphae showing localization at the tip in our slr1 deleted strain. I plan on continuing these experiments in an honors project to replicate our results to confirm whether or not Slr1 impacts the localization of She3-transported mRNAs for potential publication.

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References

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