Investigating the relationship between the RNA-binding proteins Slr1 and She3 in the fungal pathogen *Candida albicans*

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Candida albicans is an opportunistic fungal pathogen present in most of the global population that, when an individual becomes immunocompromised, undergoes a form change from a rounder, more innocuous bud-form to a filamentous hyphal-form. This filamentous form can adhere better to host human tissues and breach them, further causing disease for which mortality can be 19-24% (CDC, 2017). This form change involves the accumulation of specific proteins at the bud-tip, then the facilitation of the hyphal filament extending and adhering better to human tissues. Thus, this change can be largely attributed to differences in protein localization, for which there are two non-mutually exclusive models. The first model is that proteins are produced around the cell center, the nucleus, and shuttled to the tip. The second model, and that which is the focus for the McBride lab research, is that mRNAs, messages of code that read like a blueprint to make a protein, are transported from the nucleus to the bud-tip and then proteins are assembled there. Understanding the molecular mechanism and transport of these mRNAs in relation to this form change could provide a clearer understanding of how to prevent *C. albicans* from causing disease, in addition to understanding this transport pathway as a model for other organisms.

Past research in the McBride and other labs has provided evidence that two mRNA binding proteins, Slr1 and She3, may play key roles in the transport of such mRNAs from the nucleus to the tip. In *C. albicans*, Slr1 has been identified as a possible key player in the transport of mRNA to the hyphal tip, as it can bind RNA and a form of it is known to frequent the hyphal tip (Ariyachet et al., 2017). Further, conglomerates of proteins called protein complexes transport mRNAs to the bud tips, including in baker's yeast (*S. cerevisiae)*. In *C. albicans*, Slr1 is hypothesized to enter the nucleus, bind to specific mRNA transcripts, exit the nucleus, and bind to She3, another mRNA binding protein known to transport some mRNAs to the hyphal and bud tip (Elson, 2009). Whether Slr1 and She3 bind to each other directly or indirectly via mRNA is not completely elucidated. Our model for this transport is that Slr1 and She3 transport the mRNA to the tip, where the complex releases the mRNA and shuttles back to the cell center.

We hypothesized that Slr1 and She3 are working within a complex with each other to transport mRNA and that if we isolate the Slr1 protein from other cellular proteins, She3 should be found with it. To elucidate the interaction between Slr1 and She3, each was attached to a different protein "tag". Four *C. albicans* cell cultures were used: two that contained both proteins with tags and two that contained one protein with a tag and one without, to be used as controls. In collaboration with Eleanor Brakewood, after breaking apart the cells, I used magnetic beads that bound only to the tagged Slr1 protein, and we removed other proteins; the remaining proteins that associated with the beads were separated by size and visualized using antibodies that bind to the tags. Thus, if She3 and Slr1 are working within a complex, She3 would not be removed, but instead would remain with Slr1 and the magnetic beads and be visualized. Those cell strains lacking tagged She3 or Slr1 would show no isolated Slr1 and the cell strain lacking tagged She3 would thus also show no isolated She3. This experiment was performed on both yeast-form and hyphal-form cells to determine the relationship between these two proteins in both cell forms.

Through our summer research, we have gathered evidence that, although with a rather small percent yield, Slr1 and She3 are working within a complex to transport mRNA from the nucleus to the hyphal tip. The presence of tagged-She3 protein is reproducible, indicating that the initial isolation of Slr1 in turn isolates She3. This research, as well as the other research in the McBride lab this summer, provides a solid base for future publishable research that details more precisely the mechanism involved in the protein accumulation at the bud and hyphaltip via mRNA transport that supports strong continuous on-site protein building and accumulation. More research will be needed to solidify this result and to further elucidate whether Slr1 and She3 bind directly to each other or their complex is mediated by mRNA in both bud-form and hyphal-form cells.

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