Specificity of RNA-binding protein to transported mRNAs in *Candida albicans*
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The McBride lab seek to better understand the mechanisms of RNA transport in *Candida albicans*, a pathogenic yeast that causes a wide variety of infections in human patients (Falagas et al. 2006). With few classes of antifungal drugs to treat these sorts of infections and increasing rates of resistance to these drugs, there is a pressing need to identify new targets for potential intervention (Odds et al. 2003). RNA transport fits into the picture because it underlies the transition in cell shape necessary for the yeast to damage host cells. *C. albicans* exists in two main forms: a round budding form that easily spreads through the body and a hyphal form, with projections that can extend many times the lengths of the cell body. The transition from budding to hyphal form requires a complex mix of intracellular signals, but at its most basic level results in the transportation of molecules out to the extending tip where they are needed. Understanding how functional molecules are localized is an area of ongoing study.

My project was centered around understanding the function of RNA binding protein Slr1, a candidate in movement of mRNA to the hyphal tip. To test whether Slr1 binds to transported mRNA, we employed and IP-RT/qPCR experiment to isolate samples containing a specific protein complex. Comparing sequences that are known to be transported to those that remain in the cell body allows us to determine the specificity of a certain protein to transported RNA. If Slr1 is involved in the transport process, transported mRNAs should be found in higher quantity than non-transported mRNAs. In addition to wild type Slr1 protein, a mutant slr1 protein that binds more tightly to mRNA was also IPed and a positive control She3 protein was tested to increase validity of results. Results are reported as percent bound RNA, a measurement of how much of the total RNA in each cell was captured during the IP experiment.

A total of three complete immunoprecipitation experiments were performed over the course of the summer, with a significant amount of time spent optimizing each step of the process and confirming its repeatability. Testing for one transported mRNA (ASH1) and three non-transported mRNAs (ADE2, ACT1, and TDH3), we expect higher amounts of ASH1 in samples in which where protein binds specifically to transported mRNAs. If Slr1 or mutant slr1 binds specifically to ASH1, there should be more of this mRNA in the final sample compared to ADE2, ACT1, or TDH3. This same trend should be observed in the IPs for She3 protein to confirm the experimental setup is working. Across these three experiments, a significant increase was observed between percent ASH1 bound to She3 compared to percent bound ADE2, ACT1, or TDH3, confirming that the IP protocol was working. Although there was more ASH1 RNA bound to mutant slr1 than any other tested mRNA, this difference was only statistically significant between ASH1 and ADE2. There was no observed statistical difference between the amounts of mRNA bound by wild type Slr1 protein.

These results confirm previous work in the McBride lab and elsewhere that She3 binds to transported mRNA as part of a complex to move these molecules to their site of function (Elson et al. 2009). Although mutant slr1 seemed to bind more to ASH1 than ADE2, the lack of statistical significance with respect to the other non-transported mRNAs seems to indicate that the appearance of binding specificity is likely the result of much higher levels of ACT1 and TDH3 expression within the cell as compared with ASH1 and ADE2. The more mRNA present within the cell, the more likely it is to bind to mutant slr1. Even when the results are corrected to account for the amount of mRNA in the cell at the time of lysis, this result persists, likely indicating a nonlinear relationship between RNA concentration and binding to mutant slr1. The same trends in percent bound mRNA are observed for slr1 wild type, although at lower percent bound RNA. This result fits with previous work demonstrating that the mutant slr1 protein binds to more mRNA and is more stable, increasing the amount of mRNA in the purified sample, regardless of type (Ariyachet et al. 2017). Slr1 has many known functions in *Candida albicans* and it appears as if its function as a component of the mRNA transport complex is not lasting enough to produce statistically significant results for a variety of non-transported mRNAs.

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