RNA-binding Protein Binding Specificity to Transported mRNAs in *Candida albicans*

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The fungal opportunistic pathogen *Candida albicans* can cause a wide range of disease in humans (1). *C. albicans'* ability to cause disease is related to its capacity to switch its structure between a budding yeast form and an elongated hyphal form (2). Therefore, it is essential that we understand the mechanisms responsible for this structural shift. There are specific proteins in hyphal cells that affect growth and function, and it is important to study how these proteins get to the hyphal tip in order to understand its ability to cause disease. Some of the messenger RNAs (mRNAs), copies of genetic material that contain the information to make these influential proteins, are moved from the nucleus, the part of the cell that contains the majority of its genetic material, out to the hyphal tip to be used to make proteins on site.

Previous and ongoing work in the McBride lab has focused on characterizing a known *C. albicans* mRNA transport protein and identifying other proteins involved in mRNA transport that are linked to hyphal growth. She3 is a protein that transports mRNAs to the hyphal tip (3), and Slr1 is a protein that is proposed to associate with She3 to transport mRNAs to the hyphal tip. Slr1 localizes predominantly in the nucleus, and an altered form of Slr1 localizes to the hyphal tip (4). Removal of She3 causes a reduction in the altered form of Slr1 at the hyphal tip (4), implying a link between the two proteins. These results suggest a model where the altered form of Slr1 slows mRNA release at the hyphal tip (5). The research that I conducted this summer built on this model by working to determine whether Slr1 binds specifically to She3-transported mRNAs.

The general experimental approach was to purify Slr1-bound mRNAs and determine if Slr1 bound significantly more to transported mRNAs than to non-transported mRNAs. Slr1 tagged with green fluorescent protein (GFP) was purified, along with any RNAs bound to it. Then, reverse transcription quantitative PCR (RT qPCR) was used to determine the percentage of a particular mRNA bound to Slr1 (compared to the amount of that mRNA in the cell). This experiment was conducted with Slr1 and the altered form of Slr1 with a known She3-transported mRNA, *ASH1*, and three non-transported mRNAs, *ADE2*, *ACT1*, and *TDH3*. She3-GFP was used as a positive control to verify that the experimental conditions allowed this protein known to bind transported RNA to bind specifically to *ASH1* but not to non-transported mRNAs. GFP alone was used as a negative control to confirm that GFP is not binding RNA, and that any binding of mRNA is to Slr1, the altered form of Slr1, or She3.

We found that the positive control, She3, bound a significantly higher percentage of *ASH1* than *ADE2*, *ACT1*, and *TDH3*, while the altered form of Slr1 bound a significantly higher percentage of *ASH1* than *ADE2*, but the percentage of *ACT1* and *TDH3* bound were similar to the percentage of *ASH1* bound. Slr1 showed no significant difference in the percentage of mRNA bound between mRNAs tested, but these results are only from one trial. From these preliminary results we hypothesize that She3 does bind specifically to transported mRNAs, but that Slr1 may not bind specifically to transported mRNAs. The apparent difference in mRNA binding between the normal and altered forms of Slr1 could be because the altered form of Slr1 spends more time at the hyphal tip than Slr1. However, more replicates of these experiments are needed to make more confident conclusions as there was variability between experiments.

Future work also includes continuing to optimize our experimental protocol, testing a wider variety of transported and non-transported mRNAs that are expressed over a range of different levels in the cell, and performing these same experiments in hyphal cells.

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References

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