Comparing Normal and Altered Forms of Protein Binding as a Mechanism for Growth in *Candida albicans*

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During my summer research fellowship in the McBride lab, I studied protein binding interactions relevant for the protein-mRNA complex in order to determine mechanisms for mRNA transport in the yeast *Candida albicans*. The yeast *Candida albicans* is prevalent in the human body, and, while generally not harmful, can cause infections in immunocompromised populations. Unlike baker’s yeast, *C. albicans* can alternate between a budding form and hyphal (elongated) form that invades host cells. I studied mechanisms for protein binding, looking to determine how growth occurs in the hyphal form given that, in this extended form, the genetic material necessary to make proteins for cell growth (known as mRNA) may be concentrated at one end. Based on previous research, the McBride lab understands that proteins, specifically the proteins She3 and possibly Slr1, bind to certain mRNAs to move them to the tip of the cell to promote growth [1, 2]. We expected that these two proteins, if they were part of the same complex, would copurify in an experiment as they would be bound to each other as a mechanism for transporting genetic material from the nucleus to the tip. We therefore hypothesized that, since an altered form of the Slr1 protein (“alt”) is present in greater amounts in the hyphal tip, [1, 2] it would bind in greater amounts to She3 to move the mRNA. Therefore, our primary objective for the summer was to test whether She3 and the two forms of Slr1 bind in a complex to transport mRNA.

Our approach was to test how these proteins copurified by performing an immunoprecipitation experiment, which would allow for isolation of bound proteins using antibodies. To do this, I collaborated with Shawn Bayrd to grow cells with tagged forms of two versions of Slr1 proteins (using a “GFP” tag) and the She3 protein (with a “FLAG” tag), broke open the contents of the cell, and used magnetic beads to bind to any protein with a GFP tag. We then used blotting analysis to determine what other proteins were bound to those proteins on the beads, in experiments with both budding and hyphal form cells. Overall, our results indicate that both forms of the Slr1 protein bind to small amounts of the She3 protein in the cell. Bands are seen in a Western blot analysis that indicate the two proteins are bound to one another (Figure 1). We repeated this experiment multiple times throughout the summer in order to confirm the interaction.

As our evidence indicates that She3 and Slr1 bind to one another, the McBride lab can create a more comprehensive understanding of the complex of proteins that move mRNA to the hyphal tip. Further research can be done to indicate whether these proteins bind directly to one another or indirectly via the mRNA.

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**Figure 1.** Proteins from hyphal form cells were bound to beads, separated by size in a Western blot analysis, and then detected via antibodies specific to each tag. This figure indicates the copurification of the Slr1 and She3 proteins for both the normal and altered form of Slr1. The absence of bands in the control lanes (#1 for both blots and #4 for the She3-FLAG blot) indicates that only proteins tagged with GFP can be detected with antibody analysis, therefore showing where FLAG-tagged proteins are bound to GFP-tagged proteins.
References:

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