My summer project involved working with Professor Anne McBride to better understand the localization of mRNA-binding proteins in *Candida albicans* and how protein localization affects growth of this fungal pathogen.

*C. albicans* can exist in the human host in a yeast or hyphal form. Overgrowth of this fungus, known as candidiasis, can result in hyphal invasion and damage of epithelial and endothelial cells, leading to infection of mucosal membrane, bloodstream and internal organs (Sudbery, P.E., 2011). Hyphal elongation is characterized by polarized growth at the hyphal tips. In order for the plasma membrane to expand and new cell wall to be formed, vesicles must transport membranes and proteins to the site of growth. Secretory vesicles travel toward the tip of hyphae and accumulate in a region known as the Spitzenkörper (Sudbery, P.E., 2011).

The McBride lab is interested in examining how the protein Slr1 (SR-like-RNA-binding protein 1) may be related to hyphal formation in *C. albicans*. SR proteins are currently thought to be involved in mRNA export and splicing (Gibert, W. et al., 2001). Strains of *C. albicans* in which the Slr1 gene has been deleted exhibit problems forming hyphae. Slr1 has been found to localize predominately to the nucleus in *C. albicans*, suggesting its role as an mRNA-binding protein capable of regulating post-transcriptional processes, which may affect hyphal growth. A mutant protein tagged with a fluorescent green protein (GFP), *slr1*-6SA-GFP, in which six amino acids have been replaced with alanine, has been shown to localize to the cytoplasm near the hyphal tip, as well as to the nucleus. Localization at the hyphal tip resembles accumulation at the Spitzenkörper.

Based on these data, we hypothesized that *slr1*-6SA may be located at the Spitzenkörper. My summer project tested this hypothesis by investigating whether *slr1*-6SA localized with a Spitzenkörper protein, Mlc1 (*myosin light chain 1*), tagged with a red fluorescent protein (mCherry). In order to do this, I created mutant Mlc1 DNA tagged with a red fluorescent protein gene (mCherry) using fusion PCR and introduced this DNA into existing strains that produced GFP-tagged *slr1*-6SA. I then used fluorescence microscopy to look for co-localization of *slr1*-6SA-GFP with Mlc1-mCherry. Co-localization of *slr1*-6SA-GFP and Mlc1-mCherry would support the hypothesis that *slr1*-6SA is located at the Spitzenkörper in *C. albicans*, providing the first connection between RNA transport and secretory vesicles involved in hyphal growth.

I successfully created a DNA fragment encoding Mlc1-mCherry and integrated it into cells with *slr1*-6SA-GFP but could not detect red fluorescence of the Mlc1-mCherry protein in budding or filamenting *C. albicans* cells. Future directions include performing a Western Blot to probe for mCherry-tagged Mlc1 in order to determine if the protein is being expressed at all, or overexpressing Mlc1-mCherry in order to assess if lack of fluorescence is due to the protein being expressed at very low levels.
Faculty Mentor: Anne McBride

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References