Are protein interactions in the Candida albicans RNA transport system mediated by RNA?

Alex Miller, Class of 2026

Candida albicans is an opportunistic pathogen that inhabits the gut flora of over 50% of humans and is particularly dangerous for immunocompromised patients (Sudbery et al., 2011). *C. albicans* can switch between its budding yeast form and hyphal form, which is an important factor in its pathogenicity. For this reason, the McBride Lab has taken a particular interest in these two forms and the mechanism by which the yeast can switch between them. Prior research in the McBride Lab has investigated the mRNA transport system as a potential player in the transition between the two forms; however, little is known about the proteins involved in *Candida albicans*. Most of that research has been conducted on She3, which is implicated in mRNA transport. She3 is a protein in *Candida albicans* that localizes in the hyphal tip (McBride Lab, unpublished). Prior research has revealed that She3 is an important component of hyphal formation and invasion of epithelial cells, as in the absence of She3, RNA transport is inactivated altogether (Elson et al., 2009). In hopes of further investigating hyphal formation in *C. albicans*, the McBride Lab performed a copurification experiment with GFP-tagged She3 to find other proteins that may be involved in the She3 transport complex. In this experiment, Kate Paulsen and Poy Polcharee identified three interacting proteins of She3, which they called IPS proteins. My project this summer was to investigate the nature of the relationship between She3 and Ips2 using an immunoprecipitation assay to test whether the proteins are directly bound to one another, or if they are connected by an RNA bound to both proteins. The first challenge was to place fluorescent tags onto proteins of interest. I used the program SnapGene to design two oligonucleotides that would place a GFP (green fluorescent protein) tag onto Ips2, as well as a marker protein that enabled cells containing the protein to be grown on selective media. Then, PCR (polymerase chain reaction) was used to isolate the GFP tag, which would then be inserted into *Candida albicans*. After successfully testing for protein production, an immunoprecipitation experiment was conducted to test the potential binding of She3 and Ips2. *Candida albicans* cells containing GFP-tagged Ips2 and She3 proteins tagged with FLAG—another tag used to identify proteins—were lysed and incubated with magnetic beads that bind to GFP-tagged proteins and any proteins bound to them. This process was conducted with and without RNAse, to test whether binding between the proteins is dependent on RNA. These proteins were collected and separated on a gel. Dim bands for FLAG-tagged protein in the presence of RNAse indicated an indirect, RNA-dependent binding between the two proteins of interest. Therefore, it was found that Ips2 and She3 are likely indirectly bound.

Faculty Mentor: Anne McBride

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Anne McBride, unpublished