**Exploring the effect of messenger RNA levels on protein location in the pathogenic yeast**

*Candida albicans*

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*Candida albicans* is a commensal fungus living within many humans. However, in immunocompromised patients systemic *Candida* infections can have mortality rates of 38-50% (1). The virulence of *C. albicans* is facilitated by its ability to switch between budding yeast and elongated hyphal cell forms (2). In the hyphal form *C. albicans* is able to penetrate organ barriers and invade the interior of the human body (3). Proteins localized to hyphae drive hyphal form and function. Localization of messenger RNA (mRNA) to the site of protein function is one mechanism hypothesized to contribute to asymmetrical protein distribution (4). RNA-binding proteins aid in the localization of mRNA by attaching to an mRNA and transporting it to the correct location in the cell (5).

A detailed model of mRNA localization has been developed in the yeast *Saccharomyces cerevisiae*, focused on the mRNA *ASH1*, which plays a role in pseudohyphal development (5). In this model yeast, two cytoplasmic proteins, Khd1 and She2, enter the nucleus and, along with the nuclear protein Puf6, attach to the *ASH1* mRNA. This mRNA-protein complex moves to the cytoplasm, joins the protein She3 and is shuttled along an actin cable to the developing bud tip where Ash1 protein is translated (5). While *S. cerevisiae* is similar to *C. albicans*, one difference is the lack of a clear She2 ortholog in *C. albicans*, suggesting another protein may fill this role.

In recent years, the McBride lab has identified an mRNA-binding protein called SR-like RNA-binding protein 1 (Slr1). When Slr1 is deleted from *C. albicans*, hyphal length and virulence in a mouse model are diminished (6). Furthermore, the McBride lab has developed a mutant form of Slr1 (slr1-mut) that localizes to the hyphal tip. However, when She3 is deleted from *C. albicans*, slr1-mut is no longer found at the hyphal tip. These data suggest that (1) Slr1 is involved in hyphal development and (2) Slr1 is involved in the She3 complex. Therefore, this summer I tested the following hypothesis: if Slr1 is involved in the She3 complex that brings *ASH1* mRNA to the *C. albicans* bud and hyphal tip, then if *ASH1* mRNA is overexpressed the wild-type Slr1 normally found in the nucleus will spend more time at the bud and hyphal tip.

I began by constructing cells to use as positive controls. In *S. cerevisiae* cells with normal *ASH1* expression, Khd1 and Puf6 are localized to the cytoplasm and nucleus respectively. However upon *ASH1* overexpression, both proteins spend more time at the bud tip (7,8). Tagging proteins with green fluorescent protein (GFP) allows visualization of the proteins. The brightest spots of green fluorescence allow us to observe where the tagged protein spends the most time in the cell.

Therefore, my first experiment was developing *C. albicans* cells with GFP-tagged Puf6 and Khd1 proteins to test whether, as in *S. cerevisiae*, these proteins spend more time at the bud tip upon *ASH1* overexpression. Polymerase chain reaction (PCR) was used to amplify a GFP gene that was transformed into *C. albicans* cells. *C. albicans* cells integrated the GFP-tag into their genome through a process called homologous recombination and the resulting fluorescent-tagged proteins were visualized by epifluorescence microscopy (Fig. 1). DAPI indicates the nucleus of the cell, seen in blue (Fig 1C&G). The proximity of the green fluorescence staining to the DAPI staining in Puf6-GFP images indicates localization to the nucleus (Fig 1D). The broader green fluorescence seen in Khd1-GFP with two darker spots, one of which is the nucleus and the other is the cell vacuole, indicates that Khd1 localizes to the cytoplasm under normal *ASH1* expression. The site of the GFP-tagged proteins detected by western blotting confirmed the tag was added specifically to Khd1 and Puf6.

I next constructed DNA to direct overexpression of *ASH1* mRNA. Again, PCR allowed amplification of the *ASH1* gene and this *ASH1* DNA was inserted into a vector containing a strong promoter. This new plasmid that overexpresses *ASH1* was linearized and integrated into the genomes of *C. albicans* cells with GFP-tagged Slr1, Puf6 or Khd1. This transformation was successful on selective media. Screening for *ASH1* expression by western blot and epifluorescence microscopy will be the next phase of this research. If screening confirms successful transformation, these *C. albicans* cells will have a GFP-tagged protein of interest and overexpressed *ASH1* so that the location of proteins in the presence of various levels of *ASH1* mRNA can be examined.
References


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