Identification of mRNA Transport Protein Complexes in the Pathogenic Yeast *Candida albicans*

Samuel Burnim, 2013

The yeast *Candida albicans* is an opportunistic pathogen that can cause severe systemic infections in immunocompromised patients. One major factor contributing to *Candida albicans* virulence is its ability to elongate into a thin, filamentous hyphal cell. A set of proteins that are localized to the hyphal tip allows for proper growth, function, and invasion into the host cell (Shu and Filler, 2010). This localization could be achieved by transport of either these proteins or of the messenger RNAs encoding these proteins to the hypha (Elson et al, 2009). In *C. albicans* the She3 mRNA-binding protein assists in the localization of certain mRNA molecules to the hyphal tip (Elson et al. 2009). A mRNA transport complex including She3 and associated proteins has been intensively studied in the yeast *Saccharomyces cerevisiae*, but no She3-associated proteins have yet been identified in *C. albicans* (Elson et al, 2009; Woo et al, 2003). This project thus aimed to identify proteins that associate with She3 to transport mRNAs throughout the cell, as observed in *Saccharomyces cerevisiae*.

To separate and identify these hypothesized proteins of interest, a strain of *C. albicans* that synthesized a Tandem Affinity Purification tagged (or “TAP”-tagged) version of She3 was used. This tag allows the whole She3 complex to be isolated from cell lysates by appending a short amino acid sequence to the protein of interest. This sequence, and the associated protein complex, binds to antibody-coated beads, and may be separated out of solution through centrifugation. Following incubation of a large quantity of cell lysate containing She3-TAP in a slurry of these beads, unbound cell materials were washed away and the bound proteins removed from the beads for subsequent analysis. The purified proteins were separated by gel electrophoresis and bands that represent putative She3 complex proteins were identified. These bands were excised and the proteins therein digested with trypsin, a protease used to generate smaller peptides in preparation for proteomic analysis via Liquid Chromatography – Mass Spectrometry (LC-MS).

Two of the bands excised and prepared for LC-MS are particularly interesting candidates for members of a She3 complex. These 180 kDa and 70 kDa proteins showed the greatest enrichment in purifications from TAP-She3 lysates in comparison to control lysates in which She3 lacked the TAP tag. Comparison of LC-MS data to the *C. albicans* protein database should identify these two proteins of interest.

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**References:**

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