

Investigating protein domain localization of She3 in *Candida albicans* Songa Isaac Rwamucyo, Class of 2027

Candida albicans is an opportunistic, pathogenic, microscopic fungus that lives in human hosts and can cause a variety of common, non-life threatening diseases such as vaginal infections, to more severe bloodstream infections. *C. albicans* takes on different forms including a circular bud-shape and an elongated shape known as a hypha. It is the transition between the bud and hyphal shapes that has been attributed to *C. albicans*' pathogenicity in animal models¹.

The McBride lab investigates the protein interactions that impact the mRNA transport system important in the transition of *C. albicans* from bud to hypha. She3 is a protein that has been identified as important in this mRNA transport system, both in *C. albicans* as well as other closely related species. In *Saccharomyces cerevisiae* (Baker's yeast), She2 and She3 are important for mRNA transport from mother to daughter cells. Specifically, She2 binds to specialized RNA in the nucleus of the mother cell, subsequently binding to She3 by means of the mRNA, and finally She3 interacts with a motor protein (Myo4)

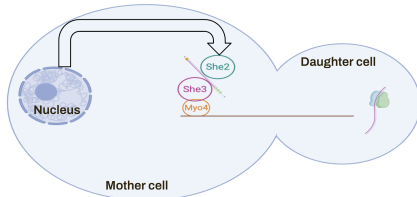


Fig 1. Diagram of mRNA transport in *S. cerevisiae* which transports this entire group of proteins to the daughter cell. In *C. albicans* She3 and a similar motor protein to Myo4, Myo2, exist, however no She2 is present. Therefore, we were interested in what specific functions She3 had in the mRNA transport system for hyphal formation in *C. albicans*^{2,3}.

In *C. albicans*, the She3 protein is made up of two domains; the front portion (N terminus) and the back portion (C terminus). We asked how these two parts of the protein affect where it is found in a hyphal cell. Due to previous literature in *S. cerevisiae* finding that the N terminus of She3 binds to Myo4, we believed the N terminus of She3 in *C. albicans* would bind to Myo2, and thus be found in the hyphal tip. Furthermore, considering the C terminus of She3 in *S. cerevisiae* binds to mRNA⁴, and the presence of a nuclear localization sequence in the C terminus of She3 in *C. albicans*, which typically directs proteins into nuclei, we expected to find the C terminus of She3 in the nuclei and generally throughout *C. albicans* hyphal cells.

To test this hypothesis we used the Green Fluorescent Protein (GFP) to visualize the N and C termini in hyphal cells using confocal microscopy.

Through PCR and Hifi Assembly we created plasmid DNA, which is a circular piece of DNA that can be put into organisms to be made by their cells, of GFP tagged N and C termini of She3. These new plasmids were then put into *C. albicans* cells and visualized under a microscope.

We found that the N terminus of She3 concentrates at the hyphal tip of *C. albicans* cells and the C terminus spreads throughout the hyphae (Fig. 2). While some concentration of the C terminus does appear to be around the nucleus (indicated by the red arrows in Fig.2), more testing must be done to confirm this.

Faculty Mentor: Anne McBride

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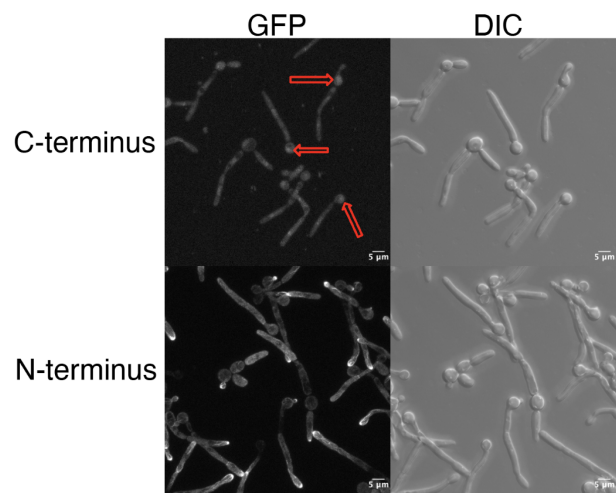


Fig 2. GFP signal of She3 N and C termini in *C. albicans* hyphal cells

¹ Mayer, F. L., Wilson, D., & Hube, B. (2013). *Candida albicans* pathogenicity mechanisms. *Virulence*, 4(2), 119–128. <https://doi.org/10.4161/viru.22913>

² Müller, M., Heym, R. G., Mayer, A., Kramer, K., Schmid, M., Cramer, P., Urlaub, H., Jansen, R. P., Niesing, D. 2011. A cytoplasmic complex mediates specific mRNA recognition and localization in yeast. *PLoS Biol.* 9(4):e1000611.

³ Elton SL, Noble SM, Solis XV, Filler SG, Johnson AD. 2009. An RNA transport system in *Candida albicans* regulates hyphal morphology and invasive growth. *PLoS Genet.* 5(9):e1000664.

⁴ Böhl F, Kruse C, Frank A, Ferring D, Jansen RP. 2000. She2p, a novel RNA-binding protein tethers ASH1 mRNA to the Myo4p myosin motor via She3p. *EMBO J.* 19(20):5514–5524.