Does the Deletion of the Ips1 Protein Impact Hyphal Tip Localization of *ASH1* mRNA in *Candida albicans?*

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The opportunistic fungal pathogen *Candida albicans* exists in both budding and filamentous hyphal forms, and the ability to switch between these two forms is crucial to its pathogenicity (Sudbery 2011). Hyphal formation, a type of polarized cell growth, results from the asymmetric localization of key proteins within the cell; one mechanism contributing to this asymmetric localization is the directional transport of mRNA to sites of protein translation at the hyphal tip (Elson et. al. 2009). The transport system underlying polarized growth in *Saccharomyces cerevisiae* (baker's yeast) is well-studied: RNA-binding proteins She3 and She2 facilitate the transport of mRNAs via the Myo4 myosin motor (Gonsalvez et. al. 2005). While *C. albicans* has a corresponding RNA-binding protein She3, the absence of She2 and Myo4 orthologs suggests that other proteins may be involved in the transport of mRNA to the hyphal tip (Elson et. al. 2009). One potential RNA-binding protein that may participate in She3-mediated transport is Interacting Protein of She3 (Ips1), which co-purifies with She3 (Pholcharee 2018). While Ips1 has been shown to localize at the hyphal tip of *C. albicans* cells during the later stages of hyphal formation, the direct importance of Ips1 in mRNA transport remains unknown (Wang 2022). This project focused on the role of Ips1 in the transport of *ASH1* mRNA, which is associated with the She3 transport system (Elson et. al. 2009). Specifically, we assessed whether the absence of Ips1 impacted *ASH1* localization to the hyphal tip. Moreover, in an effort to understand whether Ips1 plays a broader role in mRNA transport, we evaluated the localization of *RBT4*, another mRNA associated with She3, in the absence of Ips1.

To first assess the localization of *ASH1* mRNA, we used a technique called fluorescence *in situ* hybridization (FISH). This process involved growing *C. albicans* cells in an overnight culture, inducing hyphal formation, fixing the hyphae in place and removing their cell walls, and hybridizing the digested hyphae with fluorescent probes that bind specifically to *ASH1* mRNA. We then visualized the localization of *ASH1* mRNA, based on an accumulation of fluorescent signal, in cells using confocal microscopy and quantified the signal with FIJI ImageJ software. We compared the percent hyphal tip *ASH1* localization for cells with a deleted *IPS1* gene (*ips1*Δ/Δ) to cells with a deleted *SHE3* gene (*she3*Δ/Δ), cells with a deleted *ASH1* gene (*ash1*Δ/Δ), and non-modified (wild-type, or WT) cells. Our study of *RBT4* mRNA required the initial design of fluorescent *RBT4* probes, which we completed early in the summer. In the final two weeks of the internship, we modified the FISH procedure to optimize the ideal probe concentration for *RBT4* mRNA and conducted a preliminary evaluation of *RBT4* localization in *ips1*Δ/Δ, WT, *she3*Δ/Δ, and *rbt4*Δ/Δ strains.

In our examination of *ASH1* transport, we observed 5% tip localization in the *ips1*Δ/Δ strain compared to 3.2% tip localization in *she3*Δ/Δ cells and 72% tip localization in the WT strain. Since She3 is required for proper mRNA transport in *C. albicans*, the correspondingly low percentage of *ips1*Δ/Δ cells with tip localization may indicate that Ips1 is similarly important for *ASH1* mRNA transport. We intend to repeat this experiment in an effort to replicate these preliminary findings. *RBT4* probes appear to bind only to *RBT4* mRNA at an optimal concentration of 0.25 µM. Preliminary qualitative results indicate delocalized *RBT4* signal throughout the hyphal cell in both the *ips1*Δ/Δ and the *she3*Δ/Δ strains, though a formal

quantification of the signal (and subsequent repetition of the protocol) are necessary to adequately describe these findings.

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