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

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Candida albicans (*C. albicans*) is a fungus that lives commensally in human hosts. It is also an opportunistic pathogen that can cause mild to severe disease in immunocompromised individuals. The cells can exist in a budding yeast form or an elongated hyphal form. *C. albicans* has the ability to quickly switch between the two forms in response to external cues, which has been thought to contribute to its ability to become harmful to its hosts. Specifically, the hyphal form has been linked to causing infection due to its ability to invade host tissue barriers.

In its hyphal form, *C. albicans* asymmetrically localizes proteins from the mother cell to the budding daughter cell. Similarly in hyphae, RNA can be transported to the hyphal tip via an RNA transport complex. Previous studies have proposed that this mechanism is controlled by a motor protein (Elson, 2009). Studies in the McBride lab have identified a RNA transport complex with motor protein Myo2, and RNA binding protein She3, through co-purification experiments (Pholcharee, 2018). Proteins that have a high tendency to co-purify with She3 may have an important role assisting She3 during RNA transport.

This summer, I focused on the two proteins that most frequently co-purify with She3, called interacting proteins of She3 1 and 2 (Ips1 and Ips2). Ips1 is an uncharacterized protein that has been found to localize at the hyphal tip during late stages of hyphal growth (Wang, 2022). Ips2 is characterized as a RNA binding protein, but it also has a specific RNA-binding domain that is related to transcriptional and translational regulation in other proteins (Pholcharee, 2018). Studying these proteins will help further characterize their roles in *C. albicans.*

Since we hypothesize Ips1 and Ips2 are involved in She3-meditated RNA transport, my goal was to determine whether the interaction between She3 and Ips1/2 is mediated by RNA. To test this we used an immunoprecipitation (IP) assay to determine whether She3 and Ips1/2 co-precipitate in the presence and absence of RNase. We tagged Ips1/2 and She3 with two different tags in order to visualize them via immunoblot. Fortunately, a strain of *C. albicans* with tagged She3 already existed. We spent the initial stages of our project adding a tagged Ips1 or Ips2 protein to the cells with tagged She3. We used the cells containing tagged Ips1 or Ips2 and tagged She3 for the IP.

To test whether the protein interaction is mediated by RNA, we split our samples in half and treated one half with RNase to degrade any RNA in the sample. Our results confirmed previous findings, which is that in the presence of RNA (no RNase), She3 and Ips1 co-precipitate. After performing the IP in the presence of RNase, we found that She3 and Ips1 seem to co-precipitate. However, the She3 and Ips2 interaction appears to be RNase sensitive, as very small amounts of She3 co-precipitated with Ips2 in the presence of RNase. As this was the first IP experiment done for She3 with Ips1/2, we used yeast form cells since they are easier to work with than hyphae. In the future, it would be important to repeat the IPs using hyphal form cells to test protein interaction during hyphal formation. Future studies will also involve experiments to quantify the levels of protein we found to co-precipitate in presence of RNase in this experiment.

This project has allowed me to practice experimental design, important lab techniques and procedures, and literature review. All of my experiences from this summer will help complete my honors project this upcoming year.

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