Exploring the Interaction between Proteins and mRNA in the She3 Transport System

Rootjikarn Moonrinta, Class of 2021

Candida albicans is a fungus that normally lives harmlessly in association with humans. However, in an individual having an impaired immune system, it can cause a wide range of infections. *C. albicans* has two major forms, a budding yeast and an elongated hypha. The ability to switch forms is crucial for its ability to cause disease. The hypha contains proteins which aid the fungus in host cells attachment and invasion (Sudbery, 2011). One of the ways a cell could concentrate proteins at the hyphal tip is to transport messenger RNA (mRNA), genetic information used to produce a specific protein, from a site in the central part of a cell to the hyphal tip where it is used in protein production. However, the process of mRNA localization in *C. albicans* remains poorly understood. Understanding the process involved in *C. albicans*'s virulence could enable us to find a way to reduce its virulence and improve human health.

How mRNAs are moved has been extensively studied in baker's yeast, *Saccharomyces cerevisiae.* The mRNA is bound to an RNA-binding protein, She2, and form a complex with a transport protein, She3. She3 links the complex to a motor protein which transports the complex to the bud tip (Bertrand et al., 1998; Long et al., 2000; Gonsalvez et al., 2003). A similar system exists in *C. albicans* with some distinctions such as different type of motor protein and the absence of She2 (Woo et al., 2003; Elson et al., 2009). Recent work in the McBride lab has identified proteins in *C. albicans* that physically contacts to She3: a protein called Interacting-Protein of She3 (Ips1) has a region similar to proteins that bind to RNA indicating the possible function of Ips1 in mediating mRNA transport in place of She2 in *S. cerevisiae* (Pholcharee, 2018).

My project aims to investigate the ability of Ips1 to interact with a She3-transported mRNA named *ASH1* using the yeast three hybrid assay. In this assay, an RNA-protein interaction turns on a "reporter" gene present in yeast cells, which can be detected by color of colony, a group of cells divided from the same single cell, which are grown in a special growth medium (Bernstein et al., 2002). The experimental controls include other mRNA-protein pairs of known interaction from *S. cerevisiae* (She2 and She3 with *ASH1*). I will also test the interaction of *ASH1* with She3 from *C. albicans* to compare the function of She3 between the two species.

Previous studies in *S. cerevisiae* found that She2 and She3 bound to a region on *ASH1* mRNA called localization element (LE) and transport the mRNA to the bud tip (Chartrand et al., 1999). When introduced *ASH1* mRNA from *C. albicans* (*CaASH1*) to *S. cerevisiae* cells, the mRNA also localized in the cytoplasm of the budding cell indicating that *ASH1* might contain a similar LE as *ASH1* from *S. cerevisiae (ScASH1)* and binds to She2 and She3 from *S. cerevisiae* (Munchow et al., 2002). From these studies results, I hypothesize that *ASH1* mRNA might interact with Ips1 and She3 from *C. albicans* and She2 and She3 from *S. cerevisiae*. The yeast three hybrid assay would result in blue colony when cells are grown on a special growth medium.

To determine possible LEs in *CaASH1* to use in the yeast three hybrid assay, I used computer programs to compare Ca*ASH1* sequence and structure to other fungi that has She3 in comparison to *S. cerevisiae* and to predict the structure of the *ASH1* mRNA from different species. The structure of the mRNA determines its ability to bind to an RNA-binding protein and is directed by the interaction of nucleotides in the sequence. Three of four LEs from *ScASH1* had no similarity in sequence and structure with *CaASH1* and *ASH1* other fungi. However, another LE of *ScASH1* is located at the end of the mRNA (3' UTR) which is the site normally contains LE in mRNAs from other organisms (Olivier et al., 2005). Therefore, 3'UTR region of *Ca*ASH1 might also contains LE and could be used in the assay.

This summer, I did a careful reading of many scientific literature on She3 transport system and came up with a research question and experiment to answer the question. I had compiled the list of yeast strains and plasmids to use in the experiment. I also met with my mentor and lab members weekly to share ideas and discuss literature and our experiments. I hope to be able to conduct this experiment as my independent study in the Spring semester. This summer experience helps me develop my scientific thinking and deep scientific papers reading skill and allows me to work collaboratively with people who share the same interest. The experience would be beneficial for my further study in graduate school and future career as a researcher.

Faculty Mentor: Professor Anne McBride

Funded by the Robert Freedman '87, P'17 and Anne Cirillo P'17 Student Fellowship

References

Bernstein, D.S., Buter, N., Stumpf, C., and Wickens, M. (2002). Analyzing mRNA-protein complexes using a yeast three-hybrid system. Methods *26*, 123-141.

Bertrand, E., Chartrand, P., Schaefer, M., Shenoy, S.M., Singer, R.H., and Long, R.M. (1998). Localization of ASH1 mRNA particles in living yeast. Mol Cell *2*, 437-445.

Chartrand, P., Meng, X.H., Singer, R.H., and Long, R.M. (1999). Structural elements required for the localization of ASH1 mRNA and of a green fluorescent protein reporter particle in vivo. Curr Biol *9*, 333-336.

Elson, S.L., Noble, S.M., Solis, N.V., Filler, S.G., and Johnson, A.D. (2009). An RNA transport system in Candida albicans regulates hyphal morphology and invasive growth. PLoS Genet *5*, e1000664.

Gonsalvez, G.B., Lehmann, K.A., Ho, D.K., Stanitsa, E.S., Williamson, J.R., and Long, R.M. (2003). RNA-protein interactions promote asymmetric sorting of the *ASH1* mRNA ribonucleoprotein complex. RNA *9*, 1383-1399.

Long, R.M., Gu, W., Lorimer, E., Singer, R.H., and Chartrand, P. (2000). She2p is a novel RNA-binding protein that recruits the Myo4p-She3p complex to *ASH1* mRNA. EMBO J *19*, 6592-6601.

Munchow, S., Ferring, D., Kahlina, K., and Jansen, R.P. (2002). Characterization of *Candida albicans ASH1* in *Saccharomyces cerevisiae*. Curr Genet *41*, 73-81.

Olivier, C., Poirier, G., Gendron, P., Boisgontier, A., Major, F., and Chartrand, P. (2005). Identification of a conserved RNA motif essential for She2p recognition and mRNA localization to the yeast bud. Molecular and cellular biology *25*, 4752-4766.

Pholcharee T (2018) Exploring mechanisms of mRNA localization through the identification of RNA-binding protein complexes in the pathogenic fungus *Candida albicans*.

Sudbury PE (2011) Growth of *Candida albicans* hyphae. *Nat Rev Microbiol* 9:737-748. doi: 10.1038/nrmicro2636

Woo, M., Lee, K., and Song, K. (2003). MYO2 is not essential for viability, but is required for polarized growth and dimorphic switches in Candida albicans. FEMS Microbiol Lett *218*, 195-202.