Investigating the impact of Slr1 protein on mRNA transport in *Candida albicans*.

Ali Hussein, 2024

Candida albicans is an opportunistic fungus that lives harmlessly in more than 50% of the human population without causing adverse health effects. However, it can enter the bloodstream of immunocompromised individuals and result in life-threatening infections. Its infectious capability is influenced by its ability to transform between budding yeast and hyphae (elongated structures that constitute chains of cells which remain attached after division). A protein known as *ASH1* is involved in hyphae specialization in Candida and is important for its virulence (Inglis and Johnson, 2002). The *ASH1* mRNA that encodes this protein is moved to the hyphal tip before Ash1 protein is made (Elson). Therefore, exploring the transport of this mRNA plays an essential role in understanding the organism's virulence. However, *ASH1* transport has been more exhaustively explored in *Saccharomyces cerevisiae* (baker's yeast) more than Candida. In the former, a protein called She2 binds to the *ASH1* mRNA in the nucleus and moves it out to the cytoplasm where She2 and the mRNA bind to another protein called She3. She3 then collaborates with another protein known as Myo4 to move Ash1 mRNA to the bud tip. In Candida, however, She2 is not present (Elson et al., 2009). An SR-like RNA binding protein called Slr1 is considered a possible candidate to replace the function of She2 (Ariyachet 2017).

During the summer, I collaborated with Alaijah Rubianes to investigate whether Slr1 is part of the She3 complex that transports *ASH1* mRNA to the hyphal tips of Candida. We predicted that the absence of Slr1 should result in a reduced transportation of *ASH1* mRNA to the hyphal tips. We used Fluorescence *in situ* hybridization (FISH), a technique that uses fluorescent probes to visualize a specific RNA. The multi-stepped approach began with inducing hyphal formation in Wildtype and Slr1 deleted cells at 37 ºC. Then we fixed the cells and digested the cell walls by treating the cells with lyticase. After digestion, we inserted synthetic DNA probes that specifically bind to *ASH1* mRNA sequence to observe its localization. We then washed the cells and stained them with DAPI, a dye that binds to the nuclei of cells, to visualize the location of the nuclei. Finally, we observed the mRNA localization on a confocal microscope and quantified the images to produce the following results:

Experiment 1:

WT cells	***slr1Δ/Δ***
78% (71/91)	**56% (138/245)**

Experiment 2:

WT cells	***slr1Δ/Δ***
77% (63/82)	**61% (76/124)**

Fig 1. Localization of *ASH1* mRNA observed at the hyphal tips of Wildtype and Slr1 deleted cells (*slr1Δ/Δ)* in *Candida albicans* in two experiments.

The deletion of Slr1 resulted in a very little reduction of the exhibition of *ASH1* mRNA at hyphal tips. In contrast, deletion of She3 resulted in almost complete reduction of the localization of *ASH1* mRNA to hyphal tips (Elson). Therefore, since the absence of Slr1 triggers little reduction in ASH1 mRNA transport, we deduced that the RNA-binding protein is not part of the She3 complex that transports the ASH1 mRNA.

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References:

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