Pin3 – Where could you be? Pinning down the location of a prion-related protein in *Candida albicans*

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Candida albicans is a common fungal pathogen in humans that can colonize and infiltrate a majority of internal organs which can lead to life-threatening infections for people who are immunocompromised [1, 2]. Infection is linked to the transformation of *C. albicans* cells from a budding yeast form to hyphal form, a branching fiber form [3]. As a result, it is crucial to understand how this hyphal formation happens. One key protein that is responsible for normal hyphal formation and associated with virulence is Slr1, an RNA-binding protein [4]. A common approach to understanding how a protein functions is to change its shape since it is an essential aspect of normal function. More specifically, because tiny molecular interactions are necessary for proteins to function, protein structure is crucial for the essential structure of cells.

Previous students in the McBride lab discovered that a mutant Slr1 protein clumps together, forming protein "aggregates". Protein aggregation is a process associated with numerous neurodegenerative disorders [5]. However, protein aggregates can also be functional and aid in the regulation of a variety of cellular processes, including the response to stress, gene expression, memory, cell growth, and differentiation [5]. In baker's yeast, a protein called Pin3 plays a role in helping protein aggregation [6,7]. At higher temperatures, there is a higher level of protein aggregation [6,7]. Additionally, cells without Pin3 have been observed to reproduce at a decreased rate at high temperatures. In *C. albicans,* a similar protein to Pin3 binds to the mutant Slr1 protein but no further testing has been done to understand the role of this interaction until this summer.

The purpose of my research project this summer was to test whether Pin3 plays a role in protein aggregation in *C. albicans* and forms aggregates in the same place as the mutant Slr1. To test this hypothesis, I produced DNA to visualize Pin3 and integrated it into *C. albicans* cells with the help of my lab mates Izzy Lockhart and Imani Myers. The strains that we created were a green-fluorescent protein (GFP) tagged Pin3 for normal or higher than normal levels and a red-fluorescent protein (mScarlet) tagged slr1-6SA. We then imaged these strains when it was in yeast form and hyphal form at 120 and 210 minutes.

Initial results indicate that Pin3 localizes in foci for overexpressed strains compared to a more even distribution in the cytoplasm at lower levels for both yeast and hyphal form. In overexpressed Pin3 strains, Pin3 localizes at the budding tip in both yeast and hyphal forms. For normal Pin3 level strains, Pin3 localized in the cytoplasm for both yeast and hyphal forms. There were nuclear localization and cytoplasmic localization in strains with slr1-6SA-mScarlet. However, there were no cytoplasmic foci in any of the slr1-6SA-mScarlet strains for both yeast and hyphal cells. This indicates that Pin3 and slr1-6SA do not colocalize in any of the strains. Additionally, these results are different from the results that found slr1-6SA-GFP hyphal cells to display slr1-6SA-GFP localization at the tip near the Spitzenkörper of hyphae [8]. This could be caused by a longer stretch of protein linking slr1-6SA to mScarlet than to GFP, but should be investigated further. These findings contribute to the knowledge of the cellular mechanisms of *C. albicans.*

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