Investigating the localization of protein Pin3 and an associated mutant RNA-binding protein in *Candida albicans*

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My project this summer focused on locating the protein Pin3 in the opportunistic fungal pathogen *Candida albicans* in order to elucidate Pin3's function in the cell. We also wanted to see if Pin3 co-localizes with mutant RNA-binding protein slr1-mut, whose wildtype version has been implicated in *C. albicans*' ability to switch from its normal budding yeast form to a more virulent, elongated hyphal form[1]. In baker's yeast, a related organism, Pin3 induces protein aggregations, which may be helpful to the cell under stress conditions. We wanted to see whether Pin3 might perform a similar role in *Candida albicans* and whether its location in the cell could impact this role. A previous member of the McBride lab found that *C. albicans* Pin3 notably co-purifies with the protein slr1-mut[2]. We were curious if this mutant protein and Pin3 would be found together in the cell and, if so, how these proteins may interact.

To locate Pin3 in *C. albicans*, we designed two DNAs with the *PIN3* gene: one to add green fluorescent protein (GFP), and one to add GFP and increase levels of Pin3 protein. The desired DNAs were created using PCR and were ultimately transformed into cultured *C. albicans* cells. The success of the transformations was verified via immunoblot, which allows for the separation and identification of tagged proteins. Finally, fluorescence microscopy was used to visualize the GFP-tagged Pin3 protein in transformed *C. albicans* cells. Pin3 was expected to be located toward the budding tip of the cells, as seen in baker's yeast[3]. After confirming that Pin3-GFP was being produced in our cells, we constructed a DNA with the *slr1-mut* gene tagged with the red fluorescent protein mScarlet, following the same protocol used for the *PIN3* DNA. The *slr1-mut* DNA was transformed into *C. albicans* cells with and without GFP-tagged Pin3. Fluorescence microscopy was used to simultaneously visualize slr1-mut and Pin3 in our transformed cells. To see whether the yeast cells being in an elongated form affects where our target proteins are located, we induced hyphae in our transformed *C. albicans* strains and visualized hyphal form cells under the microscope.

Our microscope images indicated that when Pin3-GFP is overproduced, it forms bright foci in the cytoplasm of *C. albicans* yeast-form cells, with some inconsistent localization at the cell's budding tip (Fig.1). When Pin3-GFP is produced at normal levels, it tends to be dispersed throughout the cytoplasm (Fig.2). In cells with and without Pin3-GFP, slr1-mut was observed in the nucleus, though the tagged protein appeared brighter in cells without fluorescent Pin3 (Fig.3), meaning slr1-mut may be more concentrated in those cells. These results were consistent with localization observed in hyphal-form cells, with Pin3 being observed in bright foci at the hyphal tip in strains with elevated Pin3 levels and slr1-mut (Fig.4). No clear co-localization of Pin3 and slr1-mut was observed, tentatively indicating these proteins are not interacting. However, the nuclear location of slr1-mut suggests we may have integrated a wildtype rather than a mutant gene into the *C. albicans* genome, given that mutant Slr1 is not typically found in the nucleus[4]. The bright foci of Pin3 seen at the hyphal tip of cells overproducing Pin3 was an exciting observation, though— it could mean that Pin3 is aggregating there, perhaps with other proteins that are important for hyphal formation. It's important to note that these data are preliminary and qualitative— future directions for this research will likely include gathering quantitative data on our target proteins' localizations.

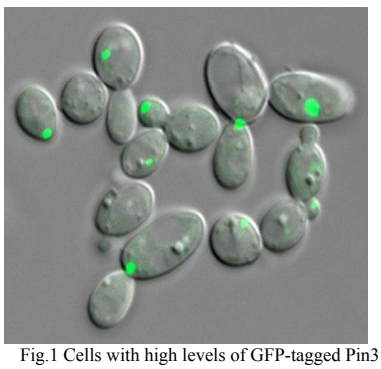

Fig.1 Cells with high levels of GFP-tagged Pin3

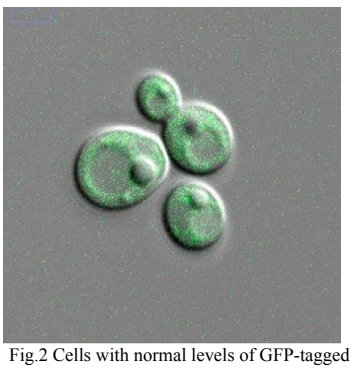

Fig.2 Cells with normal levels of GFP-tagged Pin3

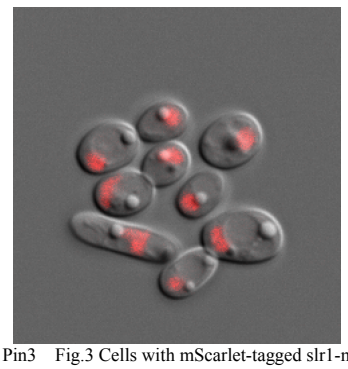

Fig.3 Cells with mScarlet-tagged slr1-mut

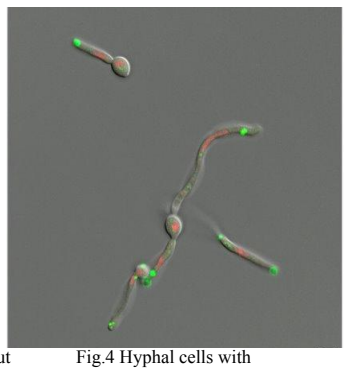

Fig.4 Hyphal cells with mScarlet-tagged slr1-mut and high levels of GFP-tagged Pin3

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References:

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3. Chernova T.A., Romanyu A.V., Karpova T. S., Shanks J.R., Ali M., Moffatt N., Howie R.L., O'Dell A., McNally J. G., Liebman S.W., Chernoff Y.O., & Wilkinson K.D. Prion induction by the short-lived, stress-induced protein Lsb2 is regulated by ubiquitination and association with the actin cytoskeleton. *Molecular cell.* 2013; *43*(2), 242-252.