

Localization of Bud3 to the septin ring during mitotic growth in budding yeast

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My research project in the Finnigan lab involved a study of the proteins that bind to the “septin” structures in budding yeast. The septin proteins are part of the cell cytoskeleton and are present in fungi and animals. They are able to form unique structures and function as barriers on the plasma membrane and also bind many other proteins. I was involved in a study of finding which proteins within yeast are able to physically interact with the septin proteins during mitosis. The septin subunits bind to each other to form linear rods that can polymerize into long filaments. These filaments can form different structures during the cell cycle. It has been found that many other cellular pathways including the cell cycle, membrane structure, and even organelles are able to pass information to and from the septin proteins. Previously, I helped screen through over 100 proteins in yeast using a split GFP method where one portion of the GFP was fused to a septin protein and the other portion was fused to a different protein in yeast. If the two proteins interacted closely within the cell, then the halves of GFP were brought close together and could assemble into a functioning protein that could produce fluorescence on the microscope. From this work, I found that the protein Bud3 is able to interact with some of the septin subunits. Bud3 is a large protein with many domains and is involved in bud site selection in yeast. I began cloning various portions of this large protein to determine which sections were required for its localization. The septin structures in yeast are found at the “bud neck” through the cell cycle. The Bud3 protein is also found at the same location. I created fusions between portions of the Bud3 protein with GFP and also fusions between one of the septin subunits (Cdc10) to the mCherry protein (to be able to see both proteins on the microscope at the same time). I used plasmid assembly techniques in yeast to make expression vectors for different portions of Bud3. From a previous study, a small amphipathic helix in the central domain of Bud3 was found to be important for its localization. I tested Bud3 constructs that either contained or were missing this small domain. For fragments of Bud3 where this helix was deleted, there was no localization to the septin structure on the microscope. Also, I found that the N-terminal domain of Bud3 was not required for localization to the septin ring during mitosis (this portion could be deleted with no effect). Therefore, our current focus is centered on this important helix as well as the C-terminal domain of this protein. Future work in the lab will generate more expression vectors to test how the Bud3 protein is able to bind to the septin ring during the cell cycle and also bind other protein partners.