Kirby Swatek, Biochemistry

University: University of Missouri, Columbia
Year in School: Junior
Hometown: St. Louis, Missouri
Faculty Mentor: Dr. Bruce McClure, Biochemistry
Funding Source: MU Monsanto Undergraduate Research Fellowship

Domain specific interactions of S-RNase binding protein with stylar 120 kDA glycoprotein in Nicotiana

Kirby Swatek, Chris Lee, and Bruce McClure

Through the process of evolution many flowering plants have developed a biochemical mechanism to prevent self-pollination and pollination by closely related plants. Gametophytic self-incompatibility (SI) is one such system that prevents inbreeding, a well-characterized disadvantage for organisms. A recently discovered protein S-RNase Binding Protein (SBP1) may be involved in SI in several species of flowering plants. SBP1 has been isolated in Nicotiana and has been shown to interact with the c-terminal of 120 kDa protein (120K), a key protein player in SI. It is unclear which domain or domains of SBP1 interact with 120K. In this experiment NaSBP1 has been cloned into pMAL-C2x an N-terminal fusion Maltose Binding Protein (MBP) expression vector. Using nucleotide primers it has been possible to amplify the desired NaSBP1 sequences for cloning into pMAL-C2x and expression in E. Coli. Transformants have been screened for expression using SDS-PAGE and western blotting. Clones that express the fusion proteins were sequenced to verify that no mutations in the DNA have occurred during PCR amplification. Binding experiments (pull-down assays) with domain specific MBP::SBP1 clones will be performed to see what domain or domains are responsible for the interaction with 120K. These experiments will provide information about which regions of NaSBP1 interact with 120K. With both individual domains and combinations of NaSBP1 domains being tested, it should be possible to elucidate which regions of SBP1 are required for the interaction. Ultimately, this experiment of the SBP1 protein will give our lab a better understanding of the structure and functionality of SI complexes in Nicotiana.