The Role of Cellulose Synthase-like D genes in tip growth of *Physcomitrella patens*

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The goal of my project was to examine the role of Cellulose Synthase-like D (CSLD) genes in tip growth of *Physcomitrella patens*. The exact function of this gene family is not yet fully understood and our research worked to investigate how these genes contribute to tip growth in the moss, *Physcomitrella patens*.

The first portion of my project focused on studying the role of Cellulose Synthase-like D1 (CSLD1) in tip growth of *P. patens*. I constructed a plasmid in which the CSLD1 gene was tagged with a green fluorescent protein (GFP). The GFP tag was used to visualize the expression of CSLD1 in living cells. I transformed the constructed plasmid DNA into wild type, Gd11, tissue. After two rounds of antibiotic selection, 16 stably transformed colonies were obtained in which GFP-CSLD1 was expressed in the wild type background. Using fluorescence microscopy, I was able to visualize the GFP-tagged CSLD1 protein expressed in living cells of *Physcomitrella patens*. We observed GFP fluorescence of CSLD1 in the plasma membrane and vesicles of the growing tips of *P. patens*. These data suggest that CSLD1 may be a contributor in proper tip growth of *P. patens*.

The second portion of my project studied single and double knockout (KO) mutants of some CSLD genes. We obtained 3 CSLD4/1, 2 CSLD5/1, and 5 CSLD6/1 double KO mutant lines from the transformation of CSLD4, CSLD5, and CSLD6 into CSLD1 KO tissue. These transformations had low yields of stably transformed colonies and these transformations will be repeated in the future for further analysis of the mutant lines and CSLD gene function. We also transformed CSLD4 and CSLD6 into wild type tissue to make single KO mutants and selection and characterization is ongoing. Further research and phenotypic analysis of these knockouts will benefit the study of the role of the CSLD gene family in *Physcomitrella patens*. 