Cell Adhesion in *Arabidopsis thaliana*

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Cellular adhesion plays an important role in plant growth and development and is thought to depend on the synthesis and structure of cell walls. Cell walls primarily consist of cellulose, hemicellulose, and pectin. Pectin is a complex polysaccharide and a major contributor to plant cell adhesion. Hence, plant cells have evolved complex mechanisms to control pectin processes. Within the Golgi, pectins are first made into polymers and then methylated by various pectin biosynthesis enzymes like QUA1, QUA2, and GAUT9. The methylated pectins are then transported inside vesicles and deposited in cell walls and the middle lamella that lies between cells. Pectin methyl esterases (PMEs) then demethylate pectin, exposing negatively charged carboxyl groups, which calcium can bind to, creating a cross-linking pectin network. The last de-methylesterification step is a tightly controlled process that regulates flexibility in plant cell adhesion and cell wall structure.

The Kohorn lab has identified several mutant plants that have reduced adhesion between cells, and through this discovered a family of Golgi membrane proteins, named ELMOs. Interestingly, only ELMO1 and ELMO4 mutant have observable phenotypes. Through creating double mutants, the Kohorn lab suspects that there is gene redundancy within the ELMO family. Hence, this summer I focused on measuring the expression level of different *ELMO* genes within wild type and mutants using quantitative PCR (qPCR) to determine if *ELMO* genes are complementing one another within mutants. We isolated RNA from 3 biological groups of wild type and mutant hypocotyls, and synthesized cDNA from the RNA templates. We designed primers against each *ELMO* gene and conducted qPCR. The results showed that all *ELMOs* are not upregulated within other ELMO mutants, except *ELMO4* is upregulated in the elmo1/2 double mutant. The elmo1/2 double mutant has the most severe phenotype of all studied mutants, and it likely needs an upregulation of another ELMO to compensate for the lost.

Besides the gene expression project, I also developed the yeast-two-hybrid assay for my honors project this fall investigating the direct protein-to-protein interaction between ELMOs and pectin biosynthesis enzymes. The Kohorn lab has found using co-immunoprecipitation, ELMO1 forms a complex with ELMO4 and three pectin biosynthesis enzymes--QUA1, QUA2, and GAUT9. The results suggest ELMOs act as a scaffold for pectin biosynthesis enzymes. However, it is unclear if ELMOs bind one or all components of this biosynthetic complex. Therefore, we obtained cDNAs for all protein of interest, used PCR and Gibson assembly to clone them into yeast two hybrid vectors. I plan to use these to test for direct protein-protein interaction this fall.

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