Final Progress Report 15 June 2015 to 14 June 2016

DOE Award Number: DE-FG02-88ER13873
Office of Basic Energy Sciences, Division of Chemical Sciences, Geosciences, and Biosciences

Regulation of Plant Cells, Cell Walls, and Development by Mechanical Signals

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Executive Summary

The overall goal of the revised scope of work for the final year of funding was to characterize cell wall biosynthesis in developing cotyledons and in the shoot apical meristem of *Arabidopsis thaliana*, as a way of learning about developmental control of cell wall biosynthesis in plants, and interactions between cell wall biosynthesis and the microtubule cytoskeleton. The proposed work had two parts – to look at the effect of mutation in the *SPIRAL2* gene on microtubule organization and reorganization, and to thoroughly characterize the glycosyltransferase genes expressed in shoot apical meristems by RNA-seq experiments, by *in situ* hybridization of the RNAs expressed in the meristem, and by antibody staining of the products of the glycosyltransferases in meristems. Both parts were completed; the *spiral2* mutant was found to speed microtubule reorientation after ablation of adjacent cells, supporting our hypothesis that reorganization correlates with microtubule severing, the rate of which is increased by the mutation. The glycosyltransferase characterization was completed and published as Yang et al. (2016). Among the new things learned was that primary cell wall biosynthesis is strongly controlled both by cell type, and by stage of cell cycle, implying not only that different, even adjacent, cells can have different sugar linkages in their (nonshared) walls, but also that a surprisingly large proportion of glycosyltransferases is regulated in the cell cycle, and therefore that the cell cycle regulates wall maturation to a degree previously unrecognized.

Progress Report

The first goal of the revised scope of work for this grant was to test the role of microtubule severing in the reorientation of the microtubule cytoskeleton (which itself directs cellulose synthase complexes). Our earlier work, and that of others, indicated that the mechanism by which microtubule arrays (and therefore cellulose orientation in the cell wall) are altered by mechanical stress was by severing of microtubules at points where they cross each other – a preferential severing of less-strained microtubules would cause orientation to the primary direction of maximal tensional stress. In the past year we created strains with microtubule reporters and the *spiral2* mutation, which increases severing rates at crossover points. As the presence of wild-type SPIRAL2 in the cell should suppress severing, the fact that wild-type cells reorient their cytoskeleton to changes in stress (such as those created by laser ablation of single cells in the shoot apex) was a puzzle. To see if increasing severing by eliminated SPIRAL2 protein had

an effect on reorientation as would be expected if our hypothesis were correct, and also to see if *SPIRAL2* is necessary for reorientation (which would support the alternative hypothesis that SPIRAL2 protein is part of a strain-dependent severing complex) we laser-ablated single pavement cells in developing cotyledons of the mutant reporter strain, and found that reorientation is faster than in wild type. This supports the original hypothesis, that increased severing leads to more rapid reorientation, and shows that wild-type SPIRAL2 protein is not necessary for reorganization of the microtubules, refuting the alternative hypothesis. Figure 1 shows one of the experiments.

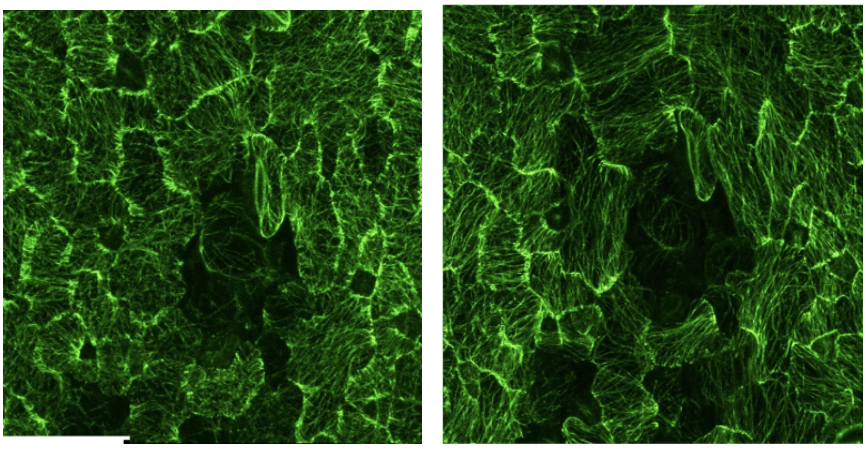

Figure 1. Left panel is a microtubule reporter line mutant for *spiral2* immediately after ablation of epidermal cells, right panel show full reorientation to the predicted stress pattern after 4 hours (in wild-type, this takes about 6 hours).

The second goal of the work done was a large-scale characterization of the primary cell walls of the shoot apical meristem. We planned (and completed) in situ hybridization to the RNAs of all glycosyltransferase genes shown by RNA-seq experiments to be expressed in shoot meristems, both to wild-type and to the *clavata3* mutant, and such experiments with additional mutants are in progress. We also planned to find the exact stage of the cell cycle in which cell-cycle expressed glycosyltransferase genes are expressed by fluorescent reporter experiments and double fluorescence in situ hybridization with cell cycle stage reporters, this also was done. We used available antibodies to observe specific cell wall linkages by immunolocalization to sectioned meristems, as proposed. This work was published as Yang et al. (2016) Regulation of meristem morphogenesis by cell wall synthases in Arabidopsis. Current Biology **26**, 1404-1415.

The published summary of the paper is:

“The cell walls of the shoot apical meristem (SAM), containing the stem cell niche that gives rise to the above-ground tissues, are crucially involved in regulating differentiation. It is currently unknown how these walls are built and refined or their role, if any, in influencing meristem developmental dynamics. We have combined polysaccharide linkage analysis, immuno-labeling, and transcriptome profiling of the SAM to provide a spatiotemporal plan of the walls of this dynamic structure. We find that meristematic cells express only a core subset of 152 genes encoding cell wall glycosyltransferases (GTs). Systematic localization of all these GT mRNAs by in situ hybridization reveals

members with either enrichment in or specificity to apical subdomains such as emerging flower primordia, and a large class with high expression in dividing cells. The highly localized and coordinated expression of GTs in the SAM suggests distinct wall properties of meristematic cells and specific differences between newly forming walls and their mature descendants. Functional analysis demonstrates that a subset of CSLD genes is essential for proper meristem maintenance, confirming the key role of walls in developmental pathways."

We continued the analysis of the meristem cell walls in collaboration with Michael Hahn and his group at the DOE Complex Carbohydrate Research Center at the University of Georgia, who have monoclonal antibodies additional to the antibodies used in the publication, by glycome profiling of wild-type and *cev1* (cellulose synthase CESA3 loss of function) mutant meristems, using antibodies to stain 4M KOH extracts of cell walls from about 300 meristems of each type. The results (Figure 2) indicated that loss of cellulose synthase activity causes substantial change in several xylan and xyloglucan polysaccharides; thus indicating that future RNA-seq and immunolocalization studies of cellulose synthase mutants are justified.

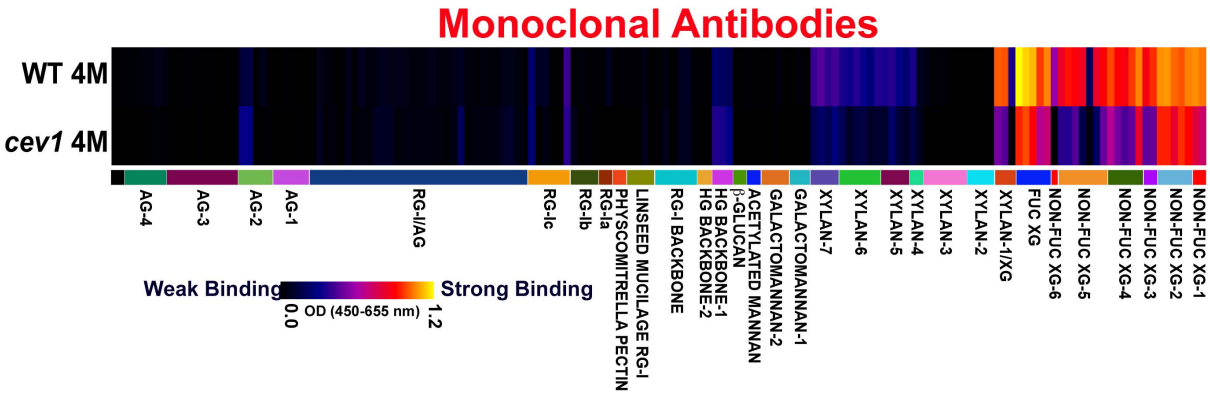

Figure 2. Representation of binding of antibodies to sugars shown at bottom, higher binding is shown as yellow and red, lower by cooler colors. Xyloglucans are at lower levels in the mutant meristems as compared to wild-type.

3) List of publications acknowledging DOE DR-FG03-88ER13873 since 6/15/15:

Yang, W., Schuster, C., Beahan, C.T., Charoensawan, V., Peaucelle, A., Bacic, A., Doblin, M.S., Wightman, R. and Meyerowitz E.M. (2016) Regulation of meristem morphogenesis by cell wall synthases in Arabidopsis. Current Biology **26**, 1404-1415.