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Mechanics of Interdigitating Morphogenesis in Pavement Cells

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Plant cells come in a wide variety of shapes, and each geometry is intimately related to the cell's respective function. How these sometimes complex cellular shapes are attained however is poorly understood. Unlike mammalian cells, the driving force for plant cell growth is provided by the internal turgor pressure, whereas spatio-temporal control of the process lies in the mechanics of the material forming the cellular envelope - the cell wall. Shaping of plant cells can therefore be regarded as a question of mechanics of the cell wall. Leaf pavement cells with their interdigitated protrusions and indents resembling a jig-saw puzzle make an ideal model to study the genesis of complex geometries in plant cells (Figure 1). Panteris and Galatis [1] speculated that a higher density of cellulose microfibrils in the neck regions of the undulating cell borders results in augmented stiffness of the cell wall thus locally restricting cell expansion. The deposition of cellulose is supposed to be regulated by the underlying microtubule cytoskeleton in the necks, while actin filaments are present mostly in lobe regions supposedly promoting wall expansion potentially through the delivery of soft pectin material to these sites. Despite this insightful hypothetical model to this date a mechanical validation has not been brought forward. A major challenge has been the visualization of cellulose microfibrils at sufficient resolution. In this project, we use confocal laser scanning microscopy and polarizing fluorescence microscopy in conjunction with in-silico mechanical modelling of the cell wall to understand the mechanical phenomena underlying the complex shaping of pavement cells.

To visualize the spatial distribution of the main cell wall components in the leaf epidermis of *Arabidopsis thaliana* seedlings we used specific labels. Calcofluor white and Pontamine Fast Scarlet 4B (S4B) confirmed the presence of localized accumulation of cellulose microfibrils in the neck regions of the pavement cells (Figure 2a). Moreover, the polarized fluorescence microscopy of S4B shows characteristic spatial configuration of the microfibrils in the neck regions (Figure 2c). These results suggest that cellulose microfibrils locally reinforce the periclinal wall in those regions. Microfibrils also descend along the anticlinal walls in these regions (Figure 2b). Staining the seedlings with propidium iodide, a fluorescent probe with affinity for pectin, in particular for the weakly esterified variety, exhibits a more intense signal on the neck sides of cell undulations (Figure 3). As mechanical stiffness of pectin is known to increase through de-esterification, we suggest that pectin dynamics might be also involved in formation of pavement cell undulations. We used these experimental data to inform a mechanical model based on finite element methods that simulates the generation of lobed cell shapes (Figure 4).

References:

[1] E Panteris and B Galatis, New Phytol. **167** (2005), p. 721-32.

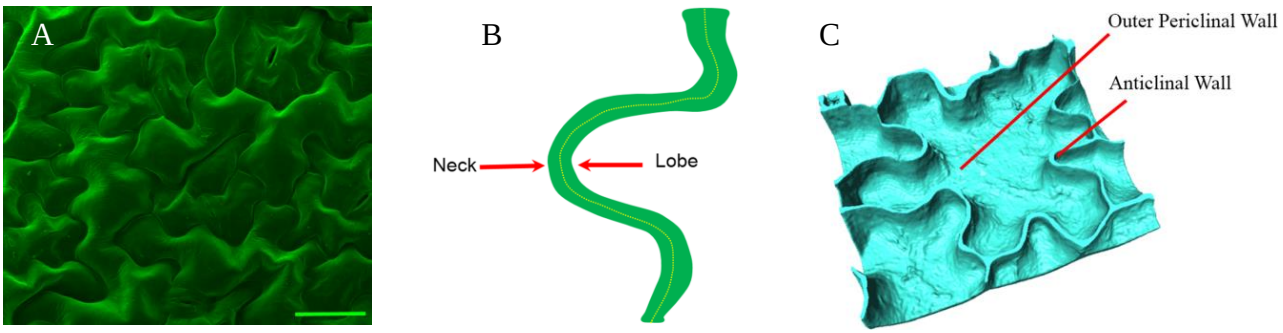

Figure 1. a) Scanning electron micrograph of *Arabidopsis* leaf pavement cells. Scale bar = 30 µm b) Definition of a pair of lobe and neck in a wall undulation c) Rotated 3D reconstruction of 3D stack of confocal micrographs of a pavement cell.

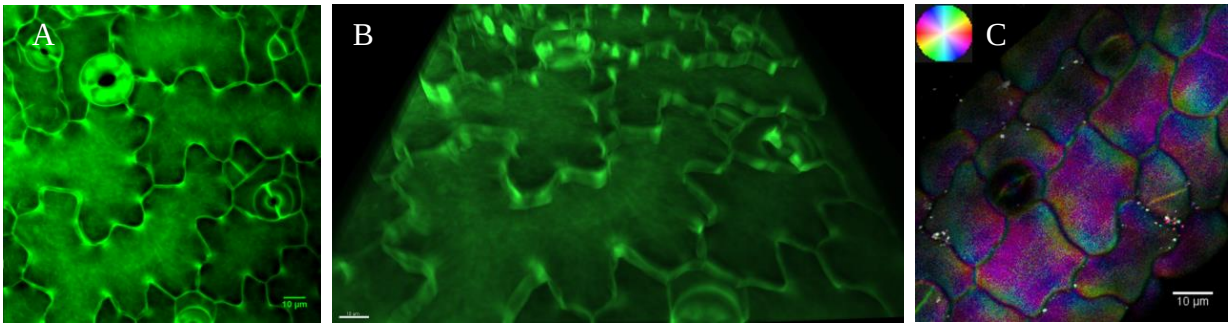

Figure 2. Calcofluor white staining demonstrates a) localized fan-shaped cellulose bundles in necks b) Extension of cellulose bundles down the anticlinal walls in undulations. c) Polarized fluorescence of S4B in the pavement cells of *Arabidopsis thaliana.* Pseudocolor-coded orientation of cellulose microfibrils reveals subcellular variations in the periclinal wall of individual pavement cells. Scale bar = 10 µm.

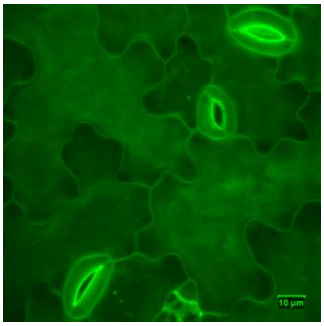

Figure 3. Propidium iodide staining shows presence of de-esterified pectin on the neck side of undulations. Scale bar = 10 µm.

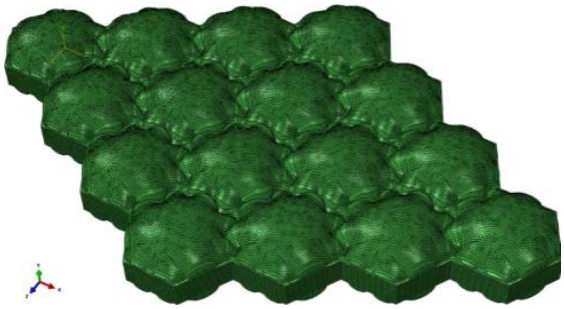

Figure 4. Finite element model of pavement cells generating undulating cell boundaries.