

Seeing the Cell Wall in a New Light¹

How cell expansion is controlled to achieve a specific cell morphology remains one of the frontier questions in plant biology. The carefully guided extension of the plant cell wall produces a remarkable variety of cell shapes, and in a larger context, creates the physical frame for the plant itself. Flowering plants generate new cells through divisions from meristematic stem cells with subsequent divisions following geometric and developmental rules (Steeves and Sussex, 1989). Each cell undergoes a lineage-specific expansion, controlled by developmental factors but strongly influenced by environmental cues. Deceptively simple shapes, like the rectangular cells found in roots and shoots, grow primarily through elongation of connected sidewalls, leaving the apical and basal cell faces almost static (Gendreau et al., 1997). More complex cell forms, like trichomes, guard cells, and the puzzle-piece-like cells found in the leaf epidermis, show combinations of diffuse expansion and polar growth (Szymanski and Cosgrove, 2009). Understanding the mechanisms directly affecting cell shape requires a multidisciplinary approach, integrating genetic and cell biological data with biomechanical studies of the cell wall. In this issue of *Plant Physiology*, Altartouri et al. (2019) introduce Brillouin microscopy to the experimental toolbox for studying cell shape formation.

Cell wall mechanics and the mechanisms driving plant cell growth have a long and rich experimental history (Baskin, 2005; Cosgrove, 2014). The dominant narrative emerging from decades of biochemical and biophysical studies places cellulose microfibrils in the principal role governing the material properties of the wall, with hemicelluloses providing crosslinks and pectic polysaccharides acting mostly as filler (Carpita and Gibeaut, 1993; McCann and Roberts, 1994). The length scale and observed coalignment of the extruded cellulose microfibrils provide a natural explanation for many of the wall's mechanical properties. Crystalline cellulose is structurally rigid and can be crosslinked into a mesh that takes on important physical properties related to the number of crosslinks, the length distribution of the fibrils, and the degree of fibril co-orientation (Eichhorn and Young, 2001). Experiments showing that perturbations to cellulose deposition produce more isotropic cell shapes led to the idea that co-oriented cellulose fibrils impart a material anisotropy to the cell wall, resulting in anisotropic cell expansion and the formation of nonspherical cell shapes (Baskin, 2005). Further discoveries showing that

microtubules at the cell cortex can influence how cellulose is patterned into the wall (Paredez et al., 2006), and that secreted proteins (e.g. xyloglucan endotransferases, expansins) alter cellulose crosslinking in response to wall acidification (Cosgrove, 1998), extended this narrative to show how plant cells could potentially orient their cellulose microfibrils and activate growth to achieve specific shapes (Cosgrove, 1998, 2016; Van Sandt et al., 2007).

Genetic screens, initially investigating how cell wall polymers are made, led to the fascinating discovery that pectins play substantial structural roles in the plant cell wall and do not simply control wall porosity (Micheli, 2001). Pectins include a narrow class of relatively short chain polysaccharides that are secreted into the wall in a soluble, methyl-esterified form. De-esterification leads to precipitation and calcium-dependent gelation in the cell wall. Pectin gels are relatively weak, compared to cellulose, and the length scale of individual molecules does not provide an obvious mechanism for creating materials with anisotropic properties. Hence, structural roles for pectins have long been doubted as significant for plant cells. Indeed, softer secreted materials (e.g. callose) are used structurally in lieu of pectins for constructing new cell plates, for extending pollen tubes, and for defense-related emergencies (Chen and Kim, 2009). Genetic manipulations of enzymes that control the degree of pectin esterification clearly show, however, that cell shape, and even anisotropic cell shapes, depend on the correct placement and chemical modification of pectic polysaccharides (Peaucelle et al., 2012, 2015).

The idea that pectins are a structural element in the plant cell wall, hypothesized to function in the genesis of cell shape, upsets a long-held narrative and provides much needed grist for a tired mill. One specific case that is certain to provide a challenge for both the new “pectinites” and the more traditional “cellulosers” to explain is how lobing occurs in leaf epidermal cells. Pavement cells start out with relatively indistinct shapes, but form interlocking lobes with neighboring cells as the leaf expands, increasing the adjoining surface area. The formation of these puzzle-piece shapes has been posited to occur through a variety of mechanisms (Panteris and Galatis, 2005; Szymanski, 2014; Sapala et al., 2018; Vőfély et al., 2019) guided by either local differences made in the strength of the anticlinal wall materials (i.e. the walls attached to each other) or the relief of stresses in the outer-facing periclinal cell walls (Fu et al., 2005; Panteris and Galatis, 2005; Armour et al., 2015; Higaki et al., 2017; Majda et al., 2017; Elsner et al., 2018). The composition and organization of both the cellulose and pectic polysaccharides change over the course of lobe initiation and maturation. How these changes relate to the mechanical properties of the cell wall remains a challenging

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question and highlights specific gaps in our global understanding of cellular morphogenesis. For example, the two adjoining pavement cells are bound together by pectins and certain hemicellulose molecules where the cell on one side cannot “grow” its cell wall without reciprocal expansion of the wall from the neighboring cell. This is a long-held tenet of the plant biology world stemming from the requirement that the cell walls form a structurally stable framework for the plant and do not slip relative to each other. Moreover, plant cells are not known to endure ingrowths from other cells that would lead to an indentation and reduction in cell volume. So how do two adjacent pavement cells expand their shared walls such that both develop lobes that appear to have grown into the neighboring cell?

Characterizing the mechanical properties of the cell wall at a cellular level is one of the larger challenges facing plant cell biologists. Cytological probes have been developed for assessing the chemical composition of fixed and, to a lesser degree, living cells (Anderson et al., 2010; Gonneau et al., 2012). However, simply understanding wall composition or relative thickness are only weak proxies for inferring mechanical properties. Traditionally, cell wall mechanics have been couched in classical stress/strain relations, with an elastic (reversible) component and a more plastic (irreversible) extension realized after a threshold strain is reached in response to the stress of turgor pressure (Cosgrove, 2016). When expressed in more than one dimension, the mechanical formulae for wall extension become quite complicated and the distribution of stresses becomes locally dependent on cell shape (Dumais et al., 2006). Even the most ambitious models developed to explore how the material properties of the wall relate to cell expansion are forced, for lack of information, to reduce these complexities to steady-state factors or parameters representing the macroscopic compliance of the wall. For a growing cell, the wall materials are straining against the stress of turgor, where the local rates of extension depend on some set of bonded carbohydrate chains dynamically altered by the act of extension, by additions of new materials, and through changes in cell wall chemistry. Not an intractable problem, but not a place for those who thought the cell wall expansion was boring (i.e. mostly wet cardboard; D.B. Sussman, personal communication).

New noninvasive methods for assessing the physical properties of walls in living cells are desperately needed. Spectroscopic methods have been applied to plant cell walls for decades, mostly in the form of Fourier transform infra-red imaging (McCann et al., 1992) and polarized light microscopy (Green, 1962). More recently, nonlinear and pump-probe methods have entered the arena (Cox et al., 2005; Mansfield et al., 2013) and, in the article from Altartouri et al. (2019), Brillouin microscopy (Scarcelli et al., 2015). This later development holds particular promise for plant biologists wanting to measure the material stiffness of the wall without physically or chemically perturbing the growing cell. Brillouin spectroscopy is related to the

more familiar Raman spectroscopy. With Raman, a range of infra-red wavelengths are used to probe the material where the majority of the light passes through or scatters without changing wavelength/frequency. However, if the energy at a particular probe wavelength matches the energy of the molecular vibrations within a material, a small amount of the energy is scattered in a different capacity, yielding photons of slightly lower or higher wavelength. A Raman spectrum, measuring light of shifted wavelength/frequency at each infra-red probe step, can therefore be diagnostic for the molecular composition of a particular material and its energy state. Brillouin spectroscopy uses light of lower energy to probe vibrations that are set up in materials interacting with the light, referred to as “phonons” in material science. Although Brillouin spectroscopy takes advantage of the same “inelastic” scattering process as Raman, leading to photons of slightly higher or lower energy than the probe beam, the meaning is substantively different. Brillouin scattering changes with the elasticity of the material and tends to shift when the material is compressed or stretched owing to the change in bulk elastic modulus (Scarcelli et al., 2015). That is a very useful tool if applied to biological materials like the lens of the eye or the wall of an expanding plant cell (Scarcelli et al., 2011, 2015; Elsayad et al., 2016). When adapted to a laser scanning microscope platform as an imaging apparatus (Scarcelli and Yun, 2007), Brillouin microscopy provides noninvasive spatial information about the relative mechanical stiffness of the cell wall in a living plant and can be used for time-course studies.

Applied to the growing epidermal cells of the eudicot leaf, Altartouri et al. (2019) demonstrate the utility of this nascent laser-based technique for accessing new information about the cell wall during lobe formation. Using the *Arabidopsis anisotropy1* mutant, shown to produce cellulose with less crystalline structure than native microfibrils (Fujita et al., 2013), Brillouin microscopy identifies regions of the cell wall with lower intrinsic stiffness than wild type, as expected for this mutant. The linearity of the measurement and the calibration from “relative” to “absolute” changes in material stiffness remain as important technical challenges. Much like any new technology, and especially when porting a laser-based spectroscopy to an optical scanning microscope, there will be new limitations to understand as well as opportunities for innovation. How the focused beam induces and reports the information about perturbations occurring mostly transverse to the beam is not well-defined and the exact interpretation of total wall stiffness relative to wall thickness, composition, and the patterned deposition of cellulose will certainly be refined in time. The initial results from Altartouri et al. (2019) suggest that the reduction in periclinal wall stiffness observed in the *anisotropy1* mutant has little effect on lobe initiation in the early leaf cells, but ultimately limits the full extension of the lobe during cell growth. Manipulating the chemical state of the pectic polysaccharides seems to have an opposite

effect, suggesting that pectic polysaccharides are more critical for the initial formation of the lobes. The new data are used to propose a two-step model for lobe initiation and maturation that is certain to spark healthy and critical debate in a field hungry for new information about how cells expand to form the fantastic array of shapes observed in our natural world.

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