Plant Cell Growth: Do Pectins Drive Lobe Formation in *Arabidopsis* Pavement Cells?

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https://doi.org/10.1016/j.cub.2020.04.007

Pectins are conventionally thought to form a gel-like matrix between stress-bearing cellulose microfibrils in growing plant cell walls. A new study proposes a more active role in driving wall expansion. How does this proposal stack up against current evidence?

The plant epidermis is attractive for studies of cell growth because it physically limits the enlargement of some organs and because its surface, composed largely of closely packed pavement cells, is accessible to examination by optical and mechanical methods [1]. Particular interest is evoked by pavement cells that become highly lobed as they grow, resulting in a jigsaw-puzzle-like cell pattern (Figure 1) that fascinates observers and raises diverse questions about cell morphogenesis, signaling and coordination between cells, wall stress and its sensing by cells, subcellular control of wall growth, the role of cytoskeletal elements, and the functional significance of epidermal patterning [2–5].

The highly lobed pavement cells of *Arabidopsis* cotyledons have been intensely studied, leading to a roll of hypotheses about lobing mechanisms. As lobes form, the anticlinal (side) walls and the periclinal (top and bottom) walls expand in spatially variable and anisotropic patterns (Figure 1) [6,7], accompanied by cytoskeletal changes [5]. Immunohistochemical studies show that changes in pectins in both anticlinal and periclinal walls are associated with lobe initiation [8–10] whereas additional reinforcement by cellulose microfibrils may restrict periclinal wall expansion [10,11]. Stiffness measurements by atomic force microscopy and Brillouin microscopy have been combined with finite element modeling to support different hypotheses about lobe initiation and growth [10–12], and the field is rife with differing conclusions [12–14].

Into this maelstrom comes a new study of *Arabidopsis* pavement cells by Haas *et al.* [15]. They report evidence for nanofibrillar pectin (specifically, homogalacturonan) at the surface of anticlinal walls of highly lobed pavement cells. Fibril width and spacing reportedly increase after genetically induced de-esterification (that is, removal of methyl esters that block carboxyl groups of homogalacturonan). The authors go on to propose a computational model in which lobe initiation and enlargement are based on the deposition, differential de-esterification, and swelling of homogalacturonan in anticlinal walls. The model describes a growth mechanism for pavement cell lobes that has no requirement for turgor pressure, wall stress or cellulose reinforcement. These are bold claims.

What are the bases for these conclusions? Haas *et al.* [15] used super-resolution microscopy (dSTORM) for immunofluorescence imaging of cotyledon sections labeled with antibodies against homogalacturonan. In some samples, the fluorescence pattern roughly resembled parallel bands that were near the plasma membrane and were oriented perpendicular to the cotyledon surface. A sketch (their Figure 5C) resembles the vertical logs in palisade walls of frontier forts. In parallel they used cryo-facture SEM of anticlinal walls to reveal surface ridges, assumed to be homogalacturonan nanofilaments. The ridges were spaced further apart on the convex surface of lobed walls compared with those on either concave surfaces or straight walls. Overexpression of a pectin methylesterase resulted in wider ridge spacing compared to another transgenic line expressing a pectin methylesterase inhibitor. These two observations were taken as evidence that homogalacturonan is present as nanofilaments that become thicker and more widely spaced upon de-esterification. This thinking drew inspiration from X-ray diffraction studies of pure homogalacturonan drawn into fibers, where highly ordered methyl-esterified chains pack more densely than do de-esterified chains saturated with calcium.

These observations and interpretations led Haas *et al.* [15] to propose a wall expansion mechanism in which enzymatic de-esterification of homogalacturonan causes lateral swelling of packed homogalacturonan nanofilaments. Selective de-esterification on one side of the anticlinal walls could lead to curving of the wall and lobe formation. This idea was extended by the proposition that anticlinal walls increase in surface area as a result of deposition of homogalacturonan and its subsequent de-esterification. This is offered as a new concept for wall growth, one in which turgor pressure, wall tensile stresses and wall reinforcement by cellulose microfibrils do not play a role. As evidence, the authors report that turgor loss (by dehydration) collapsed the outer periclinal wall but did not change cell outlines. This was taken as evidence that anticlinal walls are not stretched by turgor forces, but one must note that this assertion was not supported by quantitative data and the lobes in the representative photograph (Figure 4B) swell and shrink in a turgor-dependent manner. The authors also report that treatments to de-esterify homogalacturonan in plasmolyzed (turgor-free) cotyledons resulted in surface expansion.
These results and interpretations are sure to provoke further discussions and tests, as many questions were left unresolved and some points are at odds with the published literature. Below are three key issues.

**Distribution of Pectic Epitopes and Growth in Anticlinal Walls**
Contrasting results were reported by Majda et al. [8], who localized various pectic epitopes in cross-sections of anticlinal walls using immunogold and TEM. This approach has comparable resolution to dSTORM microscopy, but does not lend itself to 3D reconstruction. Majda et al. observed and quantified labeling of homogalacturonan in the middle lamella (the region between two adjacent anticlinal walls), whereas Haas et al. [15] detected relatively little homogalacturonan in this region. The middle lamella is known to be rich in homogalacturonan, and so the unusual labeling patterns reported by Haas et al. [15] raise questions about the completeness of their detection method, perhaps due to limited antibody accessibility. Validation of homogalacturonan conformation and distribution by other methods, for example [16], is needed to resolve this point. In this study and in another based on onion epidermis [17], homogalacturonan in periclinal walls did not have a linear nanofibrillar conformation. Why homogalacturonan would take on an unprecedented nanofibrillar organization in these anticlinal walls is an unanswered question.

The computational model by Haas et al. [15] predicts the anticlinal wall at the lobe tips to be thicker and to grow more than walls flanking the tip. In contrast, detailed subcellular measurements have shown minimal growth at lobe tips and higher growth on the flanks [7]. Thus, measured growth patterns of pavement cells seem inconsistent with the predictions of this model, which includes specific assumptions about pectin deposition rates that have not been measured.

**Physical Effects of De-esterification**
Haas et al. [15] propose that de-esterification increases the diameter and spacing of highly ordered fibrils of homogalacturonan and they equate this swelling with wall growth. One might wonder whether homogalacturonans within ordered fibrils are accessible to pectin methylesterase. However that might be, Wang et al. [18] showed that pectin methylesterase induced swelling (thickening) of onion epidermal walls but did not increase cell wall length. This swelling resulted from electrostatic repulsion of the carboxyl groups. De-esterification also reduced wall extensibility, measured in wall creep assays for expansin action. Altartouri et al. [10] reported that de-esterified pectin was associated with mechanical stiffening of periclinal walls in the neck region at an early stage of lobe formation; they proposed that such stiffening initiates lobbing by causing differential growth of the periclinal walls flanking the anticlinal walls. Loss of expansin-mediated extensibility after de-esterification [18] may also contribute to this growth asymmetry of the periclinal wall. Pectin de-esterification has wide-ranging effects on cell walls [19] and the field seems to be far from a consensus on its role in cell growth.

**Role of Cellulose, Turgor and Wall Stresses**
The ‘expanding beam’ model of Haas et al. [15] contrasts with many studies showing that plant cell growth, including leaf growth, is sensitive to turgor pressure. This sensitivity reflects, at least in part, the need for mechanical stresses to drive sliding and separation of cellulose microfibrils within growing walls, an essential step in wall enlargement [1]. If one accepts for the moment the idea that the length and shape of anticlinal walls in pavement cells is controlled by pectin deposition and swelling, we are still left with the problem of the outer periclinal wall, which is stiff, in tension, and physically connected to the anticlinal walls. Most of the periclinal wall is omitted from Haas et al.’s model, but another finite element model of complete pavement cells — using current assumptions about cell mechanics — shows that the presence of continuous periclinal walls strongly suppresses anticlinal wall bending and extension [20]. The two models start with different premises of cell mechanics. Whether the model by Haas et al. [15], with its unorthodox view of wall growth, can be made consistent with well-established ideas of turgor, wall mechanics, and growth mechanisms is a challenge for future work.

**REFERENCES**


