Adding a Piece to the Leaf Epidermal Cell Shape Puzzle

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The jigsaw puzzle shaped pavement cells in the leaf epidermis collectively function as a load bearing tissue that controls organ growth. In this issue of *Developmental Cell*, Majda *et al.* (2017) shed light on how the jigsaw shape can arise from localised variations in wall stiffness between adjacent epidermal cells.

The jigsaw puzzle shaped pavement cells in the leaf epidermis (Figure 1) have long fascinated scientists (Thompson, 1917). Early in their development, epidermal pavement cells are isodiametric and display straight cell walls, before forming wavy contours composed of alternating lobe and neck regions (Figure 1B; Fu *et al.*., 2005). How this characteristic jigsaw cell shape arises from a biomechanical perspective has remained unclear to date (Armour et al, 2016). In this issue of *Developmental Cell*, Majda *et al.* (2017) explored how such a distinct cell shape arises and whether it results from locally established cell wall properties.

Cell walls are proposed to play a key role regulating cell morphogenesis (Bidhendi and Geitmann, 2016), prompting Majda *et al.* (2017) to initially characterise leaf pavement cell shape in 16 different *Arabidopsis* cell wall mutants. By scoring each line for their pavement cell circularity and lobe number, the authors detected several that exhibited cell geometry defects. For example, overexpressing the pectin biosynthesis enzyme β-1,4-Galactan β-1,4-Galactosyltransferase (GALS1; Liwanag et al, 2012) in the transgenic line 35S::GALSYFP featured decreased pavement cell circularity, suggesting this cell wall polymer had a positive effect on lobe formation. These results led the authors to conclude that pavement cell inter-digitation appears to require cell wall remodelling and synthesis.

Next, the authors employed a modelling approach to probe whether lobe formation requires local variation in mechanical properties to be established early in the cell walls of epidermal pavement cells. Model simulations revealed that localised wall deformation that resulted in lobe formation was possible by alternating elastically stronger and weaker layers along the wall length. Importantly, lobe formation required composite walls built from two abutting layers of material with different elastic properties (Figure 1C). Moreover, the periodicity and amplitude of lobes forming across a cell wall appeared to be dependent on the size of the local cell wall mechanical heterogeneities. Indeed, bending deformation increased with increasing relative elasticity difference between the composite cell wall parts.

To test the model predictions, the authors profiled 'real' mechanical properties of epidermal pavement cells using atomic force microscopy (AFM) on ultrathin sections. AFM revealed that wall mechanical properties around the cell perimeter were highly heterogeneous. Strikingly, this heterogeneity was correlated with cell wall shape, where straight regions were less stiff than the adjacent curved regions. To test whether the correlation between wall stiffness heterogeneity and cell wall shape was causative, Majda *et al.* (2017) also analyzed a transgenic line over expressing a constitutively active version of ROP2 [*CA-rop2]. ROP2* encodes a small Rho GTPases (ROP for Rho of Plants) which organizes the network of actin filaments in the lobes

(Fu et al., 2005). In leaves of the *CA-rop2* line inter-digitation of pavement cells was almost absent. AFM revealed that mechanical properties of CA-rop2 epidermal pavement cells were more homogenous than wild type. Hence, heterogeneities in wall properties along the cell perimeter appear to be correlated with wavy cell contours.

Another important model prediction was that heterogeneities exist in mechanical properties between abutting cell walls. To validate this, Majda *et al.* (2017) employed high-resolution AFM analyses on ultrathin sections across walls of adjacent pavement cells. AFM revealed that for straight wall sections, no difference in stiffness was detected between adjacent pavement cells. However, for interdigitated sections, a gradient in wall stiffness was detectable, with the concave side being stiffer than the convex side (see schematic in Figure 1C). Hence, heterogeneities in wall properties between abutting cells also appear to be correlated with lobed shaped contours.

Pavement cells initially have straight cell walls before they expand and their perimeter forms alternating lobe and neck regions (Figure 1B; Fu *et al.*., 2005). Cell wall heterogeneities are likely to precede lobe formation. To test this, Majda *et al.* (2017) took advantage of the fact that epidermal cells exhibit stereotypical cell division patterns and shapes (Robinson *et al*., 2011), enabling them to accurately predict future positions of lobe formation. As predicted, mechanical heterogeneity was detected along the cell perimeter before wall bending was visible. Strikingly, the cell wall was less stiff where a lobe later developed, whereas it was stiffer on the sides closer to the corners.

So what causes these apparent differences in wall stiffness between adjacent pavement cells? Immuno-localisation of cell wall components revealed striking differences for two epitopes. (1,4)-β-D-galactan and (1,5)-α-L-arabinan epitopes exhibited polar localization in curved regions, but not in the straight parts of the cell walls. More specifically, both epitopes were predominantly located at the neck side of the cell wall and rarely detected on the lobe side (Figure 1C), suggesting that they were required for lobe formation. A positive role for galactan in lobe formation is supported by the pavement cell phenotype of 35S::GALSYFP overexpression lines, which exhibited decreased pavement cell circularity. In contrast, crystalline cellulose exhibited homogeneous distribution, suggesting that local differences in this major polymer did not underpin lobe formation.

Addressing how conserved the differences in distribution of galactan and arabinan cell wall epitopes were between distantly plant species represents an important question. Fascinatingly, the authors observed similar sub-cellular patterns of wall epitope distribution in leaf pavement cells of *Arabidopsis* and the early diverging angiosperm specie camphor (*Cinnamonum camphora*). Hence, the distribution of galactan and arabinan wall epitopes appears to be conserved between these highly divergent plant species, suggesting that this bio-mechanical mechanism may be widely applied across many angiosperms.

Whilst Majda *et al.* (2017) cleverly exploited AFM with mathematical modelling and *Arabidopsis* mutants to tease apart the biomechanical mechanisms controlling the evolution of epidermal cell shape, the authors' inability through technical limitations to directly quantify cell wall stiffness (other than provide relative values along or across cell walls) ultimately limited the mechanistic insights presented in this work (which could arise from a fully parameterised mathematical model, for example). Similarly, the inability to non-invasively visualise dynamic changes in sub-cellular mechanical properties of epidermal cell walls, challenged the authors to come up with clever (but indirect) solutions. Non-invasive mechanical biosensors have been developed by animal researchers based on components from their biomechanical signalling

pathways like vinculin (Grasshof et al, 2010). The availability of equivalent biosensors in plants to non-invasively image and quantify spatio-temporal changes in biomechanical forces within tissues would help transform the field. Such biosensors have revolutionised plant hormone research, uncovering novel insights into spatio-temporal changes in hormone response and abundance (Brunoud *et al.*, 2012; Larrieu *et al.*, 2015; Rizza *et al*., 2017). Developing a plant biomechanical biosensor represents the next piece in the plant epidermal jigsaw puzzle.

References

Armour, W.J., Barton, D. a, Law, a M., and Overall, R.L. (2015). Plant Cell 27, 2484–2500.

Bidhendi, A.J., and Geitmann, A. (2016). J. Exp. Bot. 67, 449–461.

Brunoud, G., Wells, D.M., Oliva, M., Larrieu, A., Mirabet, V., Burrow, A.H., Beeckman, T., Kepinski, S., Traas, J., Bennett, M.J., Vernoux, T. (2012) Nature. 482(7383), 103–106.

Fu, Y., Gu, Y., Zheng, Z., Wasteneys, G., and Yang, Z. (2005). Arabidopsis interdigitating cell growth requires two antagonistic pathways with opposing action on cell morphogenesis. Cell 120, 687–700.

Grashoff C, Hoffman BD, Brenner MD, Zhou R, Parsons M, Yang MT, McLean MA, Sligar SG, Chen CS, Ha T, Schwartz MA. (2010) Nature. 466, 263-266

Larrieu A., Champion A., Legrand J., Lavenus J., Mast D., Brunoud G., Oh J., Guyomarc'h S., Pizot M., Farmer E.E., Turnbull C., Vernoux, T, Bennett M.J., and Laplaze, L. (2015). Nature Communications, 6, 6043-6046.

Liwanag AJM, Ebert B, Verhertbruggen Y, Rennie EA, Rautengarten C, Oikawa A, Andersen MCF, Clausen MH, Scheller HV (2012) *The Plant Cell*. 24, 5024-5036.

Rizza A., Walia A., Lanquar V., Frommer W.B., and Jones A.M. (2017) Nature Plants. [Epub ahead of print] doi: 10.1038/s41477-017-0021-9.

Robinson, S., Reuille, P.B. De, Chan, J., Bergmann, D., Prusinkiewicz, P., and Coen, E.S. (2011). Science 333, 1436–1440.

Thompson, D.W. (1917). Cambridge University Press, Cambridge.

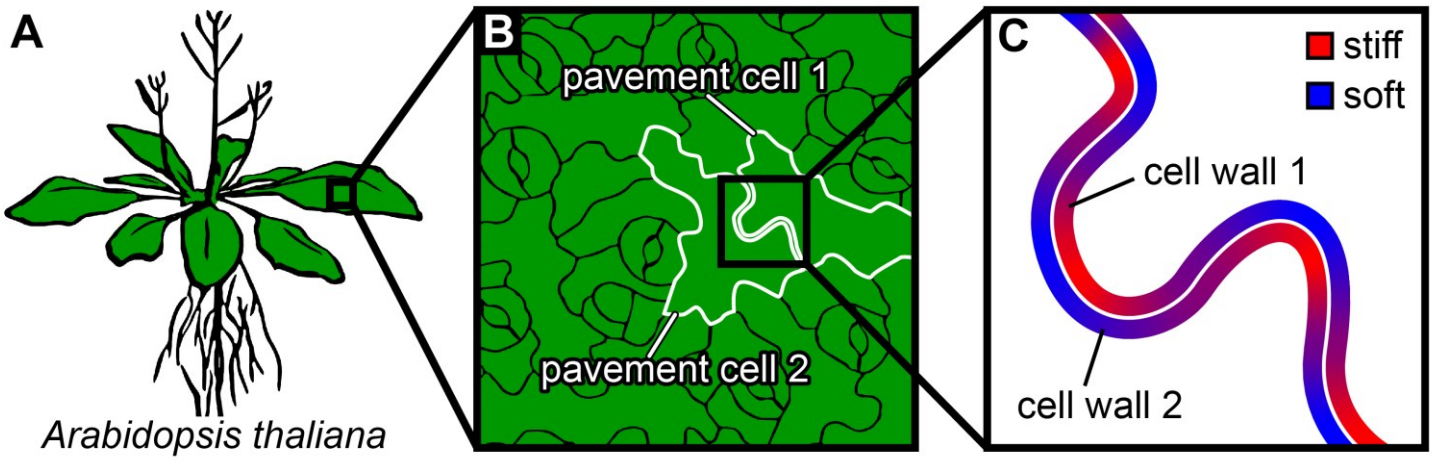

Figure 1: The shape of *Arabidopsis* leaf epidermis cells derives from heterogeneous cell wall composition. A) *Arabidopsis* plant. B) Detail of the leaf epidermis cellular patterning, C) Cell walls of two adjacent cells. Stiff cell walls (denoted in red) opposing less stiff (denoted in blue) cell walls result in the formation of wavy contours composed of alternating lobe and neck regions.