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May the Force Be with You: Overlooked Mechanical Signaling

Within multicellular plants, cells constantly communicate with their neighbors and even distant cells. A cell may produce chemical signals, such as peptides and small molecules, and perceive chemical signals from other cells. Thus, signaling chemicals mediate the exchange of information between cells. There is a simpler and faster way for cells to communicate, i.e., a cell can mechanically nudge its neighbors to send a signal. However, mechanical force is often ignored by biologists, partially due to the difficulty in directly visualizing the force, in contrast to visual detection of signaling molecules.

Although mechanical transduction between cells is possible and indeed occurs in multicellular animals, it is more efficient in multicellular plants. Plant cells are embedded in an extracellular matrix, termed cell walls, and are unable to migrate because they are effectively glued to each other. Moreover, cell death is rare in young tissues, and plant cells have high turgor pressure, the force that pushes the plasma membrane against cell walls. At a level two to ten times greater than atmospheric pressure (Beauzamy et al., 2015), plant cells and tissues are prestressed structures (Hamant et al., 2008). In fact, a cell experiences stress, which is the force per unit area, from turgor pressure and differential growth, in which slowly growing cells are stretched by fast-growing neighboring cells and vice versa. Thus, stress patterns are well suited to serve as cues for cellular behavior, including morphogenesis.

The tissue-shaping trio includes gene regulation, cellular effectors, and tissue-scale coordination. Mechanical signals are suitable for directing both cellular effectors and tissue-scale coordination. Mechanical stress leads to deformation of a solid, which is termed strain. Cell walls serve as the "exoskeleton" of cells, and the balance between turgor pressure and wall extensibility determines growth. Turgor pressure has no direction and is therefore isotropic in all directions. In contrast, wall extensibility can be anisotropic. For a given cell, subcellular wall extensibility can be highly anisotropic to enable divergence in growth.

Expanding plant cells are usually covered by primary cell walls, which consist of cellulose microfibrils embedded in a matrix of hemicellulosic and pectic polysaccharides. Cell growth symmetry breaking can be achieved by subcellular asymmetry in wall composition. For example, dark-grown hypocotyls grow asymmetrically to form apical hooks. Growth asymmetry is preceded by, and depends on, a cellular asymmetry in pectin de-methylesterification of bipolar walls. Pectin de-methylesterification in turn determines wall mechanics (Peaucelle et al., 2015).

Leaf epidermal cells present another striking example of anisotropic cell growth. Interdigitated jigsaw-shaped cells, the major type of leaf epidermal cells, are made up of alternating indentation (neck) and outgrowth (lobed) regions (Figure 1A). Such undulations are absent in young pavement cells and are established during cell maturation. How is this complex morphology established? Computational modeling suggests that alternating anticlinal walls with softer and harder elastic properties can develop into lobes and necks, respectively, when the wall is under tension. Consistent with the model prediction, the mechanical properties of the anticlinal walls of lobes and necks are significantly different. Greater amounts of arabinans and galactans, as well as low methylesterified pectin, are found in softer lobe regions, which may explain the differences in wall mechanics (Majda et al., 2017).

Anisotropic properties of the cellulose network contribute more broadly to differences in extensibility between individual walls. In contrast to short-chained hemicellulosic and pectic polysaccharides, cellulose microfibrils are long and unbranched. If we liken cell wall components to the makeup of concrete walls, hemicellulose and pectin analogously correspond to concrete, while cellulose microfibrils correspond to the steel reinforcing bars that resist tensile stress. Unlike concrete walls, cell walls are remarkable in their ability to undergo massive expansion. When many cellulose microfibrils are aligned in a particular direction, cell walls resist growth in that direction.

Interdigitated jigsaw-shaped cells also illustrate the contribution of cellulose microfibrils to anisotropic cell shape deformation (Figure 1A). Cellulose microfibrils are required for the shaping of pavement cells (Higaki et al., 2017), and the determination of cellulose microfibril orientation is an important process. The microtubule network regulates *de novo* synthesis of cellulose microfibrils by guiding the cellulose synthase complexes in the plasma membrane. A long-standing view is that microtubule orientation is generally aligned along the main tensile stress direction, aside from other factors (Green and King, 1966). Live imaging has shown that the necks and lobes create a stress pattern correlating with microtubule orientation. For example, microtubules are enriched in the neck regions with higher tensile stress. Tissue-level stress on top of the cell-shape-derived stress leads to intracellular coordination of the microtubule networks (Sampathkumar et al., 2014; Armour et al., 2015). Microtubules in turn guide cellulose microfibril biosynthesis, and potentially other wall reinforcement (Sampathkumar et al., 2014), leading to a feedback loop that reinforces the cell walls parallel to the maximum tensile stress direction. The neck regions have higher tensile stress, which aligns microtubules

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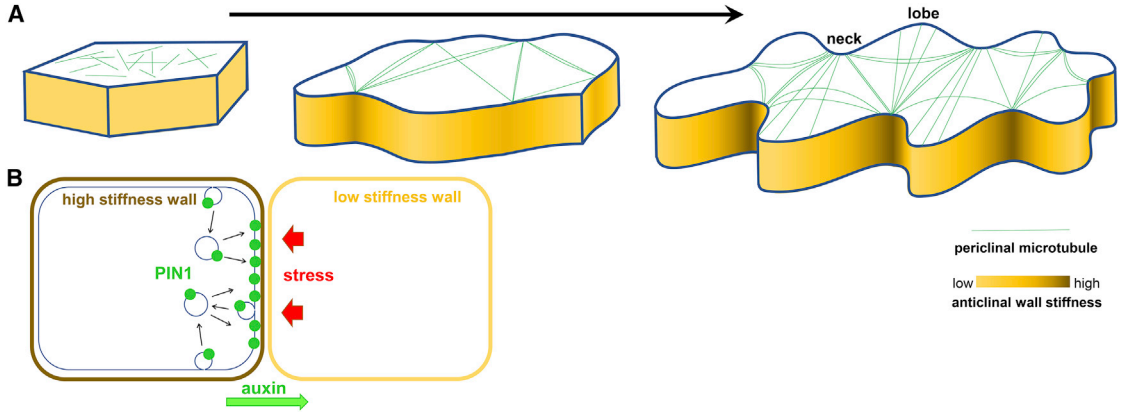

Figure 1. Mechanical Regulation of Shaping and Morphogen Distribution.
(A) Diagram of pavement cell morphogenesis, maturing from left to right. Anticlinal and periclinal walls are shown. The green lines indicate periclinally oriented microtubules, which correlate with mechanical stress patterns. The degree of anticlinal wall stiffness is indicated by the yellow gradient.
(B) The role of wall loosening on PIN1 polarization. Stress from cells with loosened walls leads to enhanced exocytosis in neighboring cells and PIN1 polarization toward the cell with loosened walls.

and microtubule-guided cellulose microfibril deposition, resulting in wall reinforcement parallel to the stress direction, which further promotes neck region indentation (Figure 1A).

Tissue growth requires tissue-wide polarity fields that coordinate cell polarity (Mansfield et al., 2018). Because mechanical stress often encompasses several cells and even an entire tissue, it likely coordinates tissue-scale patterning. For leaf epidermal pavement cells, computational modeling predicts a supracellular stress pattern that can be inferred from the microtubule networks (Sampathkumar et al., 2014). At the shoot apex, the shoot apical meristem (SAM) initiates all shoot organs. Assuming the epidermis restricts inner tissues (Hamant et al., 2008), the stress pattern is expected to be isotropic at the meristem center, around the meristem dome, and along the boundary separating lateral organs. As in pavement cells, the supracellular microtubule arrangement correlates with the predicted stress pattern (Hamant et al., 2008). Auxin maxima precede organ initials, and auxin accumulation leads to changes in cell wall properties. On the one hand, pectin de-methylesterification is locally enhanced to reduce wall stiffness (Peaucelle et al., 2011). On the other hand, the otherwise anisotropic microtubule arrays are locally disorganized by auxin, which subsequently enhances wall extensibility (Sassi et al., 2014). Together, local changes in cell wall properties at the auxin maxima may enable lateral organ initiation. Soon after leaf initiation, the symmetry of the primordium transitions from (close to) radially symmetric to bilaterally symmetric. This shaping process is accompanied by asymmetric pectin de-methylesterification and wall stiffness changes, with the abaxial (ventral) side looser at first followed by the adaxial (dorsal) side (Qi et al., 2017).

Auxin transport interacts with mechanical stress to coordinate tissue-scale patterning. Auxin maxima form successively at the periphery of the SAM to promote lateral organ primordia formation, thereby determining phyllotaxis. In this process, the plasma membrane-localized auxin efflux carrier PIN-FORMED1 (PIN1) is critical for auxin maxima formation. Strikingly, polarized PIN1 localization is coordinated in neighboring cells to cause directional auxin flow toward the auxin maxima. How can a cell sense and compare the auxin concentration of its neighbors with its own to orient PIN1 localization remains an open question. The distribution of PIN1 in the plasma membrane fluctuates dynamically through exocytosis and endocytosis. Higher plasma membrane tension leads to enhanced exocytosis and locally enriched PIN1 (Nakayama et al., 2012). Because auxin promotes wall loosening, cells with higher auxin generate local stress in the neighboring cells. Together, the above cellular processes would lead to the localization of PIN1 protein to the most highly stressed plasma membrane part, i.e., the part contacting loosened walls shared with a high auxin cell, providing an explanation for phyllotactic patterning (Heisler et al., 2010).

In short, mechanical regulation plays important roles in plants. As an emerging field, there are key questions that need to be addressed to fully understand and exploit the mechanical regulation of development and growth. We do not know how cells perceive mechanical stress, although this is an active research area and several candidates, ranging from receptor-like proteins to putative mechanosensitive channels, are proposed (Hamant and Haswell, 2017). Because mechanical stress is probably one of the most ancient types of signals, it would not be surprising if there are multiple types of mechanical sensors. In fact, microtubules, and even exocytosis complexes, may sense tensile stress, despite it may be indirect. Direct measurements of mechanical parameters, such as mechanical stress, wall plasticity, and inner cell wall elasticity, also remain challenging. However, we can calculate stress based on tissue geometry, measure epidermal wall elasticity, measure turgor pressure in limited cell types, and measure cell volume changes during growth or caused by manipulation of osmotic pressure. In this regard, the development of new methods will boost the field and our understanding of development and growth in general.

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