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Getting into shape: the mechanics behind plant morphogenesis

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The process of shape change in cells and tissues inevitably involves the modification of structural elements, therefore it is necessary to integrate mechanics with biochemistry to develop a full understanding of morphogenesis. Here, we discuss recent findings on the role of biomechanics and biochemical processes in plant cell growth and development. In particular, we focus on how the plant cytoskeleton components, which are known to regulate morphogenesis, are influenced by biomechanical stress. We also discuss new insights into the role that pectin plays in biomechanics and morphogenesis. Using the jigsaw-shaped pavement cells of the leaf as a case study, we review new findings on the biomechanics behind the morphogenesis of these intricately-shaped cell types. Finally, we summarize important quantitative techniques that has allowed for the testing and the generation of hypotheses that link biomechanics to morphogenesis.

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Introduction

The shape and growth of plant cells are dependent on the precise interplay between the cells' innate turgor pressure and the stiff yet malleable extracellular cell wall encasing the cells. The cell wall functions to counteract the immense turgor pressure and prevent the cells from bursting. During cell morphogenesis, the cell wall yields to turgor pressure, leading to the irreversible process of cell expansion. This yielding process results from cell wall loosening derived from the biochemical re-modelling of the cell wall as well as changes to the synthesis of cell wall components [1]. Localized changes to the cell wall components can lead to the diverse and specialized cell shapes seen in plants. Because of the intrinsic biological and physical properties of plant cells, turgor pressure, cell growth, and cell wall architecture lead to the inherent biomechanical forces found in plant cells and tissue.

Advances in quantitative imaging technologies combined with computational modeling and biophysical measurements have demonstrated how physical cues act as instructional signals to regulate the organization and activity of intracellular molecules which in turn regulate growth and morphogenesis [2,3]. In this article, we will focus on some recent findings on the cytoskeleton and cell wall biology that impact growth and morphogenesis in plants. We will also highlight the importance of quantitative tools that allow us to formulate and test biomechanistic hypotheses that link cytoskeleton and cell wall regulation to physical and geometrical properties.

Biophysical regulation of the cytoskeleton machinery

Within the past decade, multiple studies have confirmed that microtubule organization is influenced by both external and internal physical forces acting on plant cells at the sub-cellular and supra-cellular level [3,4,5,6,7,8,9]. Specifically, microtubules are aligned parallel to the maximal principal direction of anisotropic tensile stress existing in plant cell walls and do not align along the maximal compressive stress [4,10], evidently to synthesize similarly oriented cellulose microfibrils (CMFs) that function as the major tension-bearing component to mechanically reinforce the cell wall against the aforementioned stress. Research has demonstrated the existence of a biomechanical-microtubule feedback loop that is responsible for both cell shape maintenance [7,9] as well as organogenesis [4,6]. Mechanical forces also impact local accumulation of the hormone auxin at the shoot apical meristem through the polar localization of the auxin-efflux carrier PIN-FORMED 1 [11,12]. In addition to reducing stiffness of the cell wall, the local accumulation of auxin also promotes microtubule re-organization to facilitate outgrowth of primordia from the shoot apical meristem [13].

Recent work on sepals has shown that heterogeneous growth rates of the epidermal and precursor cells of trichomes form biomechanical stress patterns that influence microtubule organization and in turn, cell growth. Furthermore, the radially elongated socket cells surrounding the trichomes act to shield mechanical forces generated by rapidly growing trichome cells, which inturn reorganizes microtubules and modifies growth patterns. This feedback loop functions as a modulator for sepal growth and

organogenesis, ensuring organ shape and size reproducibility [14•,15]. These findings are consistent with previous work that concluded that the variability of the spatiotemporal growth patterns of individual cells within a tissue was responsible for normal sepal organogenesis [16•,17].

Several master regulators modulate microtubule dynamics in plant cells. Microtubule severing by KATANIN, for example, is influenced directly by mechanical stress [[14•],7] or via local auxin accumulation [13] (Figure 1a). Apart from this, the impact of physical forces on other microtubule regulators is less well understood. Recent work on the CLASP and SPIRAL2 proteins found that they play a role in light-dependent microtubule re-ordering by regulating the plus-end and minus-end dynamics of microtubules, respectively, in hypocotyl epidermal cells [18,19]. More importantly, CLASP has been shown to localize to cell edges with increased Gaussian curvatures, facilitating the ability of microtubules to transverse adjacent cell surfaces, demonstrating the impact of cell geometry on microtubule regulation [20] (Figure 1a). In addition to light-dependent microtubule re-orientation in hypocotyls, SPIRAL2 functions in microtubule re-orientation following mechanical perturbation in sepal epidermal cells [14•]. However, discrepancies in the temporal dynamics of microtubule response to light and mechanical perturbation are observed, it is possible that the perturbation leads to regulation of different downstream targets involved in microtubule dynamics such as SPIRAL1/SKU6 [21] and MOR1 [22] involved in regulating plus-end polymerization. Given their broad roles in microtubule regulation, it is plausible that the activity of KATANIN, CLASP, and SPIRAL2 are impacted by mechanical forces during morphogenesis.

Actin filaments also play an important role in regulating CMF deposition, which presumably influences the material properties of the cell wall. Actin perturbation was shown to affect the delivery of the cellulose-synthesizing enzymes to the plasma membrane [23,24]. More recent work has shown that actin filaments in hypocotyl cells rapidly re-organize upon exposure to an external force as little as four μN within a short time period [25]. Comparatively, microtubule re-organization at the shoot apical meristem required forces in the range of 2 mN for a much longer period of time [8]. These differences, however, could have arisen due to the fact that actin filament elongation rates are 20 times faster than that of microtubule plus end polymerization [26] or due to difference in stiffness between the two polymers [27]. The structural association between actin and microtubules [28] and the recent identification of myosin [29] and kinesin [30] motor proteins that facilitate these interactions suggest that actin could potentially be involved in mediating microtubule response to mechanical forces as well as actively participating in the mechanical reinforcement of the cell by regulating the deposition of cell wall polysaccharides and proteins. How such forces are sensed and transduced to the cytoskeleton is however unknown.

The emerging role of pectins in mechanics and morphogenesis

Our working hypothesis on shape control is based on the assumption that microtubule ordering driven by mechanical stress influences de novo synthesis of CMF via guidance of cellulose synthase complexes at the plasma membrane. Though this is widely accepted, it should be noted that direct studies on this hypothesis have not been performed. In recent years, cell wall research has hinted that pectin polysaccharides may play a predominate role in cell growth and morphogenesis [31]. In hypocotyl epidermal cells, the de-methylesterification of homogalacturonan (HG) prompts a switch from isotropic to anisotropic expansion. Because this switch precedes microtubule re-organization (from randomly-oriented to parallel transverse arrays), there is evidence that suggests symmetry breaking is not entirely dependent on microtubule organization. Only after a cell switches to anisotropic expansion do the microtubules re-orient to transverse arrays in order to deposit transverse CMFs, enhancing expansion along the established growth axis [32••]. Reduced rhamnogalacturonan-I (RG-I) content in *Arabidopsis thaliana RHAMNOSE BIOSYNTHESIS 1* (*RHM1*) mutants leads to aberrant cell expansion, resulting in helical twisting of petals and root tissue [33]. Interestingly, while most mutants with twisting phenotypes are a consequence of altered microtubule behaviours [34], the *rhm1* phenotypes are thought to be a direct result of changes in RG-I cell wall composition since *rhm1* were noted to have normal microtubule organization [33]. Biochemical pectin modifications are also known to affect cell and tissue morphogenesis by modulating cell wall stiffness and elasticity. In guard cells, changes in pectin modifications and composition (as well as CMFs) have led to changes in the biomechanics of the cell wall, disrupting stomata's ability to efficiently open and close [35,36,37]. During the dorsoventral patterning process of the leaf primordia tissue, the degree of methylesterified HG is varied within a single cell, leading to biomechanical heterogeneity of the cell wall in order to promote organ shape asymmetry [38]. Collectively, these studies clearly establish pectin as an emerging player in regulating morphogenesis; however, the synergistic interaction between pectin and CMFs during morphogenesis needs to be further resolved.

Pavement cells: paving the way to understand a complex morphogenic processes

The leaf epidermis is primarily composed of pavement cells (PCs), interdigitated jigsaw-shaped cells made of alternating outgrowth (lobed) and indenting (neck) regions (Figure 1). Because young PCs are absent of any undulations and mature into more complex morphologies, the mechanics and development behind PC morphogenesis have been of great interest. It has long been hypothesized that microtubule bundles found at the neck regions