Mechanical feedback-loop regulation of morphogenesis in plants

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ABSTRACT
Morphogenesis is a highly controlled biological process that is crucial for organisms to develop cells and organs of a particular shape. Plants have the remarkable ability to adapt to changing environmental conditions, despite being sessile organisms with their cells affixed to each other by their cell wall. It is therefore evident that morphogenesis in plants requires the existence of robust sensing machineries at different scales. In this Review, I provide an overview on how mechanical forces are generated, sensed and transduced in plant cells. I then focus on how such forces regulate growth and form of plant cells and tissues.

KEY WORDS: Cell wall, Mechanics, Microtubules, Morphogenesis, Plant, Shape

Introduction
Spatial and temporal control of organ growth is an essential feature of development. A long-standing question in developmental biology is how such morphogenetic processes are achieved. Morphogen gradients are known to regulate spatially defined cellular reactions to direct organ emergence and growth across many organisms (Tabata and Takei, 2004), and most of our knowledge on morphogenesis comes from a purely biochemical perspective of these signals. However, the process of morphogenesis is, in itself, a physical phenomenon that involves change in the geometric features of cells and organs. To obtain a mechanistic understanding of morphogenesis, information from multiple scales, ranging from molecular gene regulatory networks to mechanical properties and geometry, have to be combined.

All organisms experience mechanical forces at different magnitudes, generated either intrinsically or extrinsically and spanning across molecular- to organ-level scales. Such mechanical cues have to be actively sensed in order to respond to these mechanical forces. The mechano-response is crucial for influencing several biological processes including – but not limited to – roles in disease resistance, physiology, and the development of cells and organisms. In this Review, I discuss how the mechanical forces exerted on plant cells and organs impinge on the cytoskeleton and cell wall, which in turn feed back to regulate growth and the mechanical status of cells. I provide crucial insights on recent mechanical models of morphogenesis, and approaches used to predict and measure mechanical properties of cell wall. I do not discuss topics related to the mechanics of active movement of plants such as opening and closing of stomata, the Venus flytrap or mechanics of seed dispersal (Forterre, 2013; Woolfenden et al., 2018; Galstyan and Hay, 2018; Seale and Nakayama, 2020).

Mechanics in plants
Turgor pressure and epidermal tension
In organisms possessing walled cells, intrinsic mechanical forces come about by their ability to regulate innate hydrostatic pressure known as turgor pressure (see Glossary, Box 1). Differences in water potential brought about by changes in concentration of osmotically active molecules such as ions, carbohydrates and amino acids influence the water content of the cell, which impacts on its mechanical status (Beauzamy et al., 2014). The presence of membrane water channels (aquaporins) and cytoplasmic bridges between cells (plasmodesmata) also facilitates water movement. The turgor pressure of growing cells, such as the cells of the shoot apical meristem (SAM), is in the range of 1 MPa (Beauzamy et al., 2015). In addition to developmental stage and environmental conditions, the size and local topology of the cells also contributes to heterogeneity in turgor pressure (Long et al., 2020).

As plant tissues are multilayered with cells attached to each other, the increase in turgor pressure of cells in the inner layers results in a build up of tensile forces at the outer epidermal layer. This has been illustrated in tissues such as stems and the hypocotyl, in which surgical cuts result in the gaping of the tissues when placed in water (Kutschera and Niklas, 2007). This is also the case for flat-tissue types such as cotyledons, in which the inner cell layers are not tightly packed and are frequented by air pockets. In addition to cuts that result in gradual opening up of the cotyledon, ablation of the outer epidermal cells alone (using an ultraviolet laser pulse) causes an upward displacement of the inner spongy mesophyll cells (Sampathkumar et al., 2014).

Turgor pressure drives growth
In plants, turgor pressure is resisted by the presence of a multi-lamellate, rigid – yet malleable – cell wall (Fig. 1). While counterbalancing turgor pressure, plant cell wall experiences mechanical tension around ~10 MPa or higher in plane (Beauzamy et al., 2015). Sustained tensile stretch along cell wall leads to elastic (reversible) and plastic (irreversible) or partially viscoelastic and partially viscoplastic deformation of the cell wall (Cosgrove, 2018a) (see Glossary, Box 1). Cell growth occurs by wall loosening (Cosgrove, 2018a) (see Glossary, Box 1). Wall loosening is mediated by the activity of enzymes present in the cell wall that decrease turgor pressure by reducing the tensional forces in the cell wall, which primes the cell back to a state that is competent for water uptake, and allows for further build up of turgor pressure and tensional forces.

A seminal model for plant morphogenesis was first derived on the premise that, at constant turgor pressure, the rate of cell wall yielding, which results in cell volume increase, must be equal to the rate of water uptake (Lockhart, 1965). Over the years, this model has been modified to take into consideration wall stress relaxation due to wall loosening, elastic deformations, changes in turgor pressure and...
Box 1. Glossary

Compliance. The ability of a material to be rapidly deformed upon application of forces. It is the inverse of modulus. Strain resulting from applied tensional forces can be elastic compliance (reversible) or plastic compliance (irreversible). Softer materials have greater mechanical compliance.

Elastic. A material that is readily deformable and able to recover immediately to its initial state when relieved of the forces acting on it. For an elastic material, stress and strain are directly proportional to each other.

Modulus. A measure of the material property or stiffness that is derived from the stress and strain curve. A material that has higher values of modulus are considered to be stiff.

Plastic. At low values of applied stress, solid materials exhibit elastic properties. When stress exceeds a certain limit, termed yield threshold, a plastic material yields and is unable to return to its initial state upon release of the stresses.

Strain. The ratio of change in length of a material to its original length as a result of an applied tensional force. It is also termed deformation.

Stress. Force acting on a unit area. A material with cross-sectional area (A) is brought under uniaxial tension by the application of axial pulling force of a certain magnitude (F) in opposing directions. For such a material, tensile stress is represented as σ=F/A.

Turgor pressure. Hydrostatic pressure exerted in cells that are at more than the normal atmospheric pressure.

Viscoelastic. A material that takes more time to deform upon application of stresses, as well as return to its original state when released.

Viscoplastic. A material that is plastic, but takes longer to respond to the applied force and to reach its resting state when forces are relieved.

Wall loosening. The ability of the plant cell wall to expand irreversibly when held at a constant force. This is also referred to as wall creep and requires the presence of molecular factors such as expansins. The activity of these agents results in relaxation of existing tensile stresses in the cell wall.

Wall softening. The ability of the cell wall to deform upon application of a mechanical force. This is a reversible process.

water loss due to transpiration (Cosgrove, 1985; Proseus et al., 1999; Ortega, 2010), concepts explained in exceptional detail by Geitmann and Ortega (2009). When experiencing such high tensile forces, the outermost periclinal wall of epidermal cells (parallel to the surface of the cell) is the major tension-bearing face of the cell wall (Kutscher and Niklas, 2007). The outer periclinal cell walls of most epidermal cells are thicker than their anticlinal walls (perpendicular to the cell surface), as well as the cell walls of inner cell layers, suggesting that the interplay between turgor pressure and the mechanical properties of the periclinal cell wall dominates growth-related processes in plant cells (Kutscher and Niklas, 2007). More recently, the chemical status of the cell wall has been shown to promote cell enlargement independently of turgor pressure, albeit this does not take into consideration the activity of cell wall-loosening enzymes already present in the cell wall (Haas et al., 2020).

Cell wall composition contributes to mechanics

The mechanical properties of the cell wall rely on the structural organization of its different components. The three main cell wall components that contribute to its mechanics are cellulose microfibrils (CMFs), hemicellulose and pectin. Modifications to the cell wall, either by enzymes or by chemical treatments, produce distinct responses: the irreversible process of wall loosening or the reversible processes of wall softening (see Glossary, Box 1) that results in the reduction of stiffness (Cosgrove, 2018a). Quantitative assessment of these mechanical properties has allowed us to understand the relative contribution of the different cell wall components and their interaction with the overall mechanics of the cell wall (Box 2).

Cellulose and hemicellulose

Among the different components of the cell wall, the most significant tension-bearing components are the cellulose microfibrils (CMFs), which are composed of linear chains of β-1,4-linked glucose monomers. The CMFs are arranged as multi-lamellate sheets embedded in gelatinous matrix of pectin (see below; Fig. 1). The mechanical property of the CMF is influenced by the degree of crystallinity (structured cellulose). The primary wall of growing plant cells contains amorphous CMFs, meaning it is less crystalline in nature and is therefore capable of promoting cell expansion (Thomas et al., 2013; Park et al., 2013). Consistent with this, loss of cellulose synthesis reduces cell wall stiffness and causes isotropic (non-directional) expansion of cells (Fagard et al., 2000; Zhang et al., 2019). Fine nanoindentation-based topological imaging of the inner face of a fresh periclinal cell wall shows that the spacing of the CMF is uneven, with frequent points of direct contact with adjacent CMF within each lamella (Zhang et al., 2014) (Box 2; Fig. 1). The hydrophobic face of CMFs is coated with xyloglucan, a hemicellulose component, which could potentially influence interactions between adjacent CMFs (Cosgrove, 2018b). Both cellulose and xyloglucan in extracted cell walls can be hydrolyzed by the addition of the Hypocrea jecorina endoglucanase Cel2A, which causes wall loosening without much change to the appearance of the cell wall (Yuan et al., 2001; Zhang et al., 2019), an effect that is reduced in xyloglucan biosynthesis mutants (XXT1 and XXT2) (Park and Cosgrove, 2012b). A similar result is also obtained when xxt1 xxt2 mutants are treated with α-expansin, which also promotes wall loosening, suggesting that both Cel2A and α-expansin have similar targets (Park and Cosgrove, 2012a). Solid-state nuclear magnetic resonance (NMR) spectroscopy has confirmed α-expansin targets, such as xyloglucan, are enriched in structurally distinct regions of CMFs (Wang et al., 2013). These so-called ‘biomechanical hotspots’ are formed by CMF-CMF interactions and, in certain cases, interactions with xyloglucan, which could mediate wall loosening by the activity of α-expansin (Cosgrove, 2018a). Quantitative assessment of cell wall mechanics in mutants of cellulose synthesizing enzymes have shown softening of the cell walls at the SAM (Sampathkumar et al., 2019). Sequential removal of CMF lamellae using enzymes on isolated patches of cell wall also results in softening and increased tensile compliance (see Glossary, Box 1) of the cell wall. More recently, the activity of xyloglucan endotransglucosylase/hydrolase XTH3 promotes the transglycosylation between cellulose and xyloglucan, resulting in cellulose-xyloglucan hybrid polymers in vitro (Shinohara et al., 2017). Such activities, if present in vivo, would further complicate cell wall mechanics.

Pectin

In addition to cellulose and hemicellulose, pectins (galacturonic-acid-rich polysaccharides) also influence the mechanical properties of the cell wall. As well as providing support for CMFs, pectin is a major constituent of the middle lamella that cements cells to their neighbors (Daher and Braybrook, 2015). This association ensures, for the most part, that plant cells do not slip or slide during growth and that the transduction of mechanical forces throughout the epidermis of the tissue is more homogeneous. Perturbation to pectin synthesis results in the loss of cell adhesion, thereby disrupting the transmission of tensional forces across the tissue and altering the local distribution of stress (Verger et al., 2018). The major pectin component is homogalacturoran (HG), and is made up of linear
polymers of 1,4 linked α-D-galacturonic acid. NMR spectroscopy of fresh cell walls has shown a close association between cellulose and pectin (Wang et al., 2015). Wall loosening is also enhanced in xxt1/xxt2 mutants when treated with pectin-degrading enzymes, suggesting that pectin substantially contributes to the cell wall mechanics of the mutant plants (Park and Cosgrove, 2012a). HGs are synthesized in a methyl-esterified state in the Golgi, and are selectively modified to their de-methyl-esterified form by enzymes in the cell wall. The presence of seven to 20 contiguous galacturonic acid residues in the polysaccharide backbone promotes calcium-mediated binding with adjacent pectin polymers (Voragen et al., 2009).

Several studies have reported that de-methyl-esterified HGs are associated with stiffer cell walls (Bou Daher et al., 2018); however, contrary to this, nanoindentation experiments have shown that domains of cells at the SAM containing de-methyl-esterified pectin are softer (Peaucelle et al., 2008, 2011). The authors of this latter study propose that the limited availability of calcium at the SAM might result in softer walls, despite the majority of pectin being de-methyl-esterified. However, blocking the plasma membrane calcium channels, which prevents entry of calcium into the cell from the cell wall, abolishes cytoplasmic calcium spiking events, indicating that the cell wall of SAM cells indeed contain calcium, albeit in potentially low amounts (Li et al., 2019). Alternatively, the activity of pectin-modifying enzymes could provide substrates for enzymes such as pectate lyases, which would result in softer cell walls. Although the nanoindentation measurements do indicate that cells have much softer cell walls due to the presence of de-methyl-esterified pectin, these measurements do not reveal if this is sufficient to induce wall loosening. The conclusion that these effects arise from wall loosening is based on the results from the transient activation and inhibition of the de-methyl-esterification process, which results in ectopic production and complete abolishment of organs at the SAM, respectively. Wall softening is not sufficient to promote loosening, as shown by nanoindentation and tensile stretching of isolated cell walls that are subjected to enzymatic treatments (Zhang et al., 2019). This indicates that the growth-related changes observed in the SAM from altering the methyl-esterification status of pectin might be due to activation of the cell wall integrity-sensing mechanism. More rigorous testing of...
isolated cell wall fractions has indeed shown that de-methyl-esterification results in cell wall softening by increasing plastic compliance without influencing the elastic compliance (Boxes 1 and 2) (Wang et al., 2020). As cell wall polymers are heterogeneous polyelectrolytes, this softening effect comes about by the swelling of the wall due to enhanced electrostatic repulsion between pectin carboxylates. Despite the softening effect, pectin de-methyl-esterification does not result in cell wall loosening. Super-resolution imaging of antibodies that selectively bind to pectin depending on its methyl-esterified state shows that pectin is distributed in a linear fashion along the anticlinal wall of pavement cells, rather than being highly dispersed (Haas et al., 2020). However, the masking effects of other polymers has not been investigated in detail. Furthermore, scanning electron microscopy indicates the presence of filamentous structures of similar dimensions as that of antibody labelling. Based on previous X-ray diffraction studies performed in vitro, the study proposes that HGs exist as quaternary structures (Walkinshaw and Arnott, 1981). The study also proposes that de-methyl-esterification of HGs causes a change in conformation of its quaternary structure, resulting in expansion of the filamentous structure, as opposed to the electrostatic repulsion-based swelling shown previously (Wang et al., 2020). As pectin is found in close proximity to cellulose, the authors speculate that pectin and cellulose could co-exist in such higher-order structures, which in turn has important implications in regulating the mechanics of the cell wall.

Translating mechanical signals: the microtubule-cellulose feedback loop

Predicting stress distribution patterns

The mechanical properties of the cell wall impact the shape of cells and organs, thereby modulating the distribution of innate mechanical forces. To understand how such mechanical forces could feed back to further regulate cell and organ shape, it is necessary to gain better insights into molecular elements that are influenced by mechanical forces. This first requires the evaluation of how mechanical forces in cells and organs are distributed. Finite element models (FEMs) allow for the prediction of mechanical features of cells and organs of a particular shape (Box 3). FEM using cylindrically shaped cells predicts circumferential tensile stress (hereby referred to as ‘stress’) that is twice as high as the axial stress, with the strain direction being perpendicular to the stress (Green, 1964; Bozorg et al., 2014) (see Glossary, Box 1). In puzzle piece-shaped epidermal pavement cells, the stress is highly anisotropic in the indenting domains, compared with the protruding and more central regions of the cells (Sampathkumar et al., 2014) (Fig. 2A). Stress magnitude is also spatially variable, with higher levels of stress observed in the indenting regions compared with the protrusions. Evaluation of such stress patterns has revealed that the open regions of the periclinal face of the cell also experience high stress (Sapala et al., 2018). FEMs have been applied to investigate the mechanics of pollen tubes, trichomes and guard cells (Fayant et al., 2010; Yanagisawa et al., 2015; Woolfenden et al., 2017). The extension of these models to tissue-scale levels has allowed the prediction of how cellular features contribute to tissue-level processes, and vice versa (Hamant et al., 2008; Robinson et al., 2017).

Box 2. Measurement of mechanical properties of the cell wall

Most techniques that are commercially available provide information on the ability of the cell wall to readily deform upon application of a certain force. Such a response is present in both actively growing cells and in cells that have fully matured, and does not necessarily indicate the capability of a cell to grow. The widely used atomic force microscopy (AFM), based on micro- and nanoindentation approaches, measures the resistance to the applied force in a direction normal to the plant cell wall surface, providing a measure of the compliance of the cell wall (Peaucelle et al., 2011; Milani et al., 2011; Sampathkumar et al., 2014; Zhang et al., 2019). However, it should be noted that this method is sensitive to aspects such as geometry of the sample and turgor pressure. In addition to such measurements, AFM also generates a height map of the probed surface, providing high-resolution data of cellulose microfibril organization. Iterative axial extension of the cell wall provides information on the elastic compliance. This is obtained during the first extension, after which the material is relaxed; the second extension and relaxation will provide a different response because it involves the plastic component of the material. From this it possible to estimate the plastic compliances (Zhang et al., 2019). Wall loosening is measured by using isolated patches of cell wall that are clamped and extended upon application of constant tensile forces in the plane of the cell wall. This results in the stabilization of the length of the cell wall, after which the activity of wall loosening proteins present in the wall induces wall expansion. These methods have their own limitations, as stretching of isolated patches of cells depends on the nature of cells adhering to each other. Furthermore, nanoindentation of live cells is complicated by the resistance caused by turgor pressure and hydraulic movements occurring upon indentation.

Box 3. Finite element models

One of the most widely used approaches to predict mechanical properties of cells and tissues is by the use of finite element model (FEM) (Bidhendi and Geitmann, 2018; Kennaway and Coen, 2019). FEMs use a mesh or physical structure that is subdivided into a finite number of elements connected together at vertices termed nodes. There are several variations in FEMs with two-dimensional thin plates or shells having elements that are triangles or quadrilaterals. The three-dimensional FEM contain elements that are four-node tetrahedral, five-node pyramids, six-node prismatic or eight-node hexahedral forms. These elements can be further subdivided to generate smooth deformed surfaces. By using such meshes, it is possible to study the effects of applied loading forces on meshes that have defined mechanical properties and boundary conditions. The displacement of the nodes allows for the estimation of stress and strain. FEM-based models also provide insight into how some shapes can be achieved (Bidhendi and Geitmann, 2018; Ali et al., 2014). This requires that the material properties of the mesh are spatially heterogeneous and therefore deform in defined directions when a load is applied. This deformation results in a change in the initial state of the material, which then has to be re-meshed and adjusted back to its initial state. Iterative application of these two steps would result in certain macroscopic changes to the structure of the initial mesh. However, the material properties of the meshes used for the FEM are much simpler and mostly two dimensional, and thus are not accurate representations of the actual three-dimensional architecture of the plant cell wall. Furthermore, cell walls in most FEMs are considered to be of linear elasticity, whereas plant cell walls are complex with different degrees of anisotropy, and exhibit highly nonlinear elasticity as well as complex irreversible deformations. This simplification of the mesh has created ambiguities in interpretation of data such as the anticlinal wall-dominated morphogenesis mechanisms present in pavement cells. Different quantitative approaches that measure various mechanical parameters of the cell wall have started to provide such information and can be exploited to develop models that integrate the full complexity of the cell wall (Zhang et al., 2019; Wang et al., 2020). FEMs that integrate this level of detail do exist; however, they are restricted to studying changes occurring only at the nanoscale (Dyson et al., 2012) and extending this level of scale to higher length scales representing cell and tissues is necessary.
These forces into biochemical information is necessary to bring about functional activity. Turgor pressure generated in cells is non-directional; therefore, an additional level of control that regulates cell wall organization is necessary to generate a particular shape. The earliest evidence that structural elements of cells could facilitate this is based on the observation of highly ordered transverse patterns of stiff CMFs in cylindrical cells (Green, 1969). These patterns of CMFs resemble the predicted pattern of the principal direction of stress. A shift to disorganized CMFs from the predominant transverse pattern results in isotropic growth during normal development of cylindrical cells in *Hydrodictyon africanum* (Green, 1969). Consequently, it has been shown that the organization of CMFs is similar to the arrangement of microtubules found in transmission electron micrographs (Ledbetter and Porter, 1963). Drugs that disrupt microtubule cytoskeleton result in random organization of CMFs, causing cells to grow isotropically (Green, 1962). These studies have established the foundation for how mechanical forces correlate with the structural elements of cells and in turn regulate directional cell growth (Green, 1999). It is now well established that microtubules influence the trajectories of the cellulose-synthesizing enzymes at the plasma membrane (Paredez et al., 2006). Such an association appears to be strong in epidermal cells that are experiencing high tensile forces, whereas in the inner layers CMF patterning in the SAM does not correlate with the microtubule ordering seen by field emission scanning electron microscope (Sampathkumar et al., 2019). This difference could potentially be due to the sample preparation procedures employed during such imaging procedures. Alternatively, the regulation of proteins that influence cellulose synthase complex association with microtubules, such as CELLULOSE SYNTHASE INTERACTING PROTEIN 1, might be different. The lack of adequate live cell imaging techniques to visualize such processes in inner cell layers is a limiting factor for investigating such discrepancies in cellulose synthase-microtubule interactions. It is also known that cellulose synthase complexes persist and migrate along the plasma membrane even after complete depolymerization of microtubules (Paredez et al., 2006). Migration of these cellulose synthase complexes that are not associated with microtubules is influenced by their interaction with pre-existing CMFs (Chan and Coen, 2020). The consequences of such events, and discrepancies in CMF-microtubule alignment, suggest that microtubule organization does not fully mirror aspects of wall mechanics.

The mechanical stress-microtubule-cell wall nexus

FEMs, in combination with various molecular techniques, have now established an association between mechanical stress, microtubules and CMF deposition in many cell and tissue types (Yanagisawa et al., 2015; Bou Daher et al., 2018; Sampathkumar et al., 2019). These studies demonstrate that cell and tissue geometry relies on microtubule organization and microtubule control over the cell wall machinery. Mechanical perturbation also elicits a microtubule response without causing major changes in cell or tissue geometry (Hamant et al., 2008; Sampathkumar et al., 2014; Robinson and Kuhlemeier, 2018; Verger et al., 2018). For example, physical ablation and compression of cotyledon pavement cells uncouples the cell geometry dependence of microtubule organization, resulting in a coordinated response across several cells (Jacques et al., 2013; Sampathkumar et al., 2014). The response of microtubules to such mechanical perturbations is facilitated by the activity of the microtubule-severing protein KATANIN (Uyttewaal et al., 2012; Sampathkumar et al., 2014). Furthermore, KATANIN-mediated severing at microtubule crossover sites is proposed to be restricted by

![Image of micrographs of pavement cell (A) and shoot apical meristem (B), showing distribution of the largest principal tensile stress distribution patterns.](image)

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**Fig. 2. The largest principal tensile stress distribution.** (A,B) Micrographs of pavement cell (A) and shoot apical meristem (B), showing distribution of the largest principal tensile stress-distribution patterns. Highly anisotropic stresses (red double-headed arrows) are present in the periclinal face of the indenting regions of pavement cells. Isotropic stress patterns (black double-headed arrows) are observed in the most open regions of the pavement cells (A). In the shoot apical meristem (B), the central domain experiences non-directional stresses (black double-headed arrows), whereas the peripheral region have circumferentially distributed anisotropic stress (blue double-headed arrows) and the boundary domains between the meristem and the emerging primordia also contain anisotropic stresses (red double-headed arrows).

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2011; Hervieux et al., 2017; Fox et al., 2018; Ali et al., 2019). Abstraction of cellular-scale FEM to a continuous sheet of material with defined cell scale information is also used to discretize tissue-level details for simulation purposes (Bozorg et al., 2014; Hervieux et al., 2017; Kennaway and Coen, 2019). For example, the FEM of dome shaped *Arabidopsis* SAM exhibits an isotropic stress at the central domain, a circumferential stress pattern in the peripheral region and a highly anisotropic stress at the boundary domain along the boundary crease (Hamant et al., 2008) (Fig. 2B).

**The cellulose-microtubule link**

Although a picture of how mechanical force distributions are present in cells and tissues is possible with FEM, the transduction of these forces into biochemical information is necessary to bring...
SPIRAL2 in epidermal cells of leaves and petioles, and the lack of SPIRAL2 results in a hyper-active response to mechanical perturbations in sepal cells (Hervieux et al., 2016; Wightman et al., 2013). However, it should be noted that evidence of SPIRAL2-mediated suppression of severing at crossover sites is not observed in hypocotyl cells, suggesting an indirect effect on severing due to its microtubule minus-end stabilizing activity (Nakamura et al., 2018; Fan et al., 2018). Recently, NIMA-related microtubule-associated kinase 6 has been shown to promote depolymerization of a subset of microtubules that aligns along the stresses. This dampens the microtubule response to mechanical forces, thereby limiting drastic growth differentials between cells in tissues (Takatani et al., 2020).

It is still unclear, however, how mechanical forces are transduced to the microtubule network. Recently, microtubule-based structures have been proposed to play the role of tension sensors in plants (Hamant et al., 2019), based on the observation that physical confinement of relatively stiff microtubules to a specific three-dimensional space results in the emergence of certain patterns (Mirabet et al., 2018). However, the presence of a cell wall-plasma membrane-microtubule continuum is more compelling (Hamant et al., 2019), because the transduction of mechanical forces requires the presence of a solid medium that has a network-like property to ensure transmission of mechanical forces over a specific distance (Hamant et al., 2019; Fruleux et al., 2019). As discussed above, the pre-tensed CMFs are organized as lamellate sheets and form an interconnected network-like structure; thus, CMFs serve as ideal candidates for transmitting such mechanical signals (Cosgrove, 2018b) (Fig. 1). The nascent CMFs are also linked to the existing cell wall scaffold, while being attached to the cellulose-synthesizing complex that physically interacts with cortical microtubules via scaffolding proteins such as CSI1, CMU1 and CMU2 (McFarlane et al., 2014) (Fig. 1). The transmission of forces to the microtubule network could potentially modulate the microtubule lattice architecture, which could act as binding sites for proteins such as KATANIN that locally modify the microtubule network (Diaz-Valencia et al., 2011).

**How do plants sense mechanical forces?**

Recent advances in plant mechanobiology have started to reveal the role of plasma membrane-localized proteins involved in mechanosensing (Fruleux et al., 2019). The transmission of mechanical forces at the plane of the plasma membrane is much simpler than the complex three-dimensional network of the cell wall. Therefore, there are direct consequences of structural modifications of these proteins present at the plasma membrane. In addition, some receptors present at the plasma membrane are also physically tethered to the cell wall, making them competent to sense changes in mechanical strain occurring at the cell wall.

**Mechanosensitive channel of small conductance-like protein**

Stretch-activated or force-gated mechanosensitive ion channels embedded in plasma membrane allow for the exchange of ions and osmolytes across the plasma membrane (Hamant and Haswell, 2017). Such gating processes occur due to the build up of tensional forces along the plane of the plasma membrane, which thins the membrane and opens the channel (Fig. 1). Of the different mechanosensitive channels, mechanosensitive channels of small conductance-like (MSLs) are anion-preferring ion channels that respond to changes in membrane tension. There are ten MSL proteins found in Arabidopsis, of which the majority localize to the plasma membrane. Indeed, MSL9 and MSL10 are located at the plasma membrane, and electrophysiological studies have confirmed that these are the two major MS channels found in root cells involved in the conductance of chloride ions (Haswell et al., 2008). Although the lack of these two proteins eliminates the majority of the channel activity, there are no phenotypic abnormalities under normal or mechanically challenged conditions. Conversely, the pollen-specific plasma membrane-localized MSL8 is crucial for pollen survival upon osmotic shock. In addition to being less viable during rehydration assays, msl8 mutants have pollen germination defects, with the pollen tubes bursting, indicating that MSL8 proteins act as osmotic safety valves (Hamilton et al., 2015). The gating mechanisms mostly converge on generating changes in calcium fluxes, which have profound effects on the physiology and development of plants (Monshausen and Haswell, 2013). However, conclusive evidence of the participation of MSL in generating calcium fluxes has not been reported.

**Reduced hyperosmolarity-induced calcium increase 1 protein**

In addition to MSL, reduced hyperosmolarity-induced calcium increase 1 (OSCA1) is involved in the gating of calcium ions under osmotically stressed conditions (Yuan et al., 2014). OSCA1 is a plasma membrane-localized protein belonging to a family of 15 genes in Arabidopsis, with homologues found across eukaryotic kingdoms (Murthy et al., 2018). OSCA1 proteins exist as dimers with structural similarities to other membrane-localized calcium-activated chloride channels. Mutants of OSCA1 are sensitive to osmotically challenged conditions and a related member of the same gene family, OSCA3.1, is upregulated upon exposure to dehydration stress (Kiyosue et al., 1994). Electrophysiological studies of Arabidopsis OSCA1 expressed in mechanically insensitive animal cell lines, results in the activation of the channel on application of pressure (Zhang et al., 2018; Murthy et al., 2018). Together, these findings show conclusive evidence for the involvement of OSCA proteins in mechanotransduction.

**Mid1-complementing activity protein**

Mid1-complementing activity (MCA) protein is another candidate mechanosensitive ion channel, initially obtained in a screen for identifying suppressors of the yeast mid1 mutant, which lacks mechanically-gated calcium permeation. Electrophysiological measurements, as well as over-expression studies of MCA proteins, have revealed changes to calcium fluxes after mechanical stimulations (Nakagawa et al., 2007). Arabidopsis loss-of-function MCA mutants lack competence to penetrate hard agar media and exhibit defects in lignin production upon cellulose perturbation (Denness et al., 2011). However, they do not structurally resemble the yeast MID1 or any known ion channels, suggesting that MCA could play a role in regulating the activity of another calcium channel.

**Rapidly activated calcium channel activity/DEK1 protein**

More recently, membrane patches derived from calli have shown the presence of small fluxes of calcium ions upon the application of pressure. The activity of an unidentified rapidly activated calcium channel activity (RMA) channel is dependent on the presence of DEFECTIVE KERNEL1 (DEK1) (Tran et al., 2017) (Fig. 1). DEK1 is the only plant member of a calpain family of calcium-dependent cysteine proteases (Lid et al., 2002). Calcium-dependent autocatalytic cleavage of DEK1 results in the uncoupling of the active calpain region from the transmembrane domain-containing part of the protein. In mammalian systems, calpains are involved in several signaling pathways that include integrin receptor-linked adhesion motility and shear stress induced motility (Croall and
Ersfeld, 2007). In *Arabidopsis*, DEK1 regulates the expression of genes involved in epidermal cell fate specification and maintenance, and therefore has an influence on the function of cells and tissues (Malivert et al., 2018). In addition, DEK1 also influences microtubule organization, cell wall composition and cell adhesion, which presumably impacts on the transmission of tensional forces in plants (Amanda et al., 2016). Although most mechanosensitive channels that are calcium permissive exhibit mild phenotypes when mutated, loss of *dek1* is embryonic lethal, suggesting that DEK1-mediated RMA activity plays a significant role in mechanosensing in plants. It is also known that calpains act downstream of mechanosensitive channels such as PIEZO in animals, suggesting an evolutionary convergence between plants and animals.

To date, different mechanosensitive ion channels have been described, yet how they relate to each other is unclear. Variation in structure, subcellular localization and expression patterns of these channels suggest that some of the channels might have evolved to sense and respond to different degrees of stresses or, in some cases, function in processes independent of mechanical sensing.

**Receptor-like kinases**

Apart from mechanosensitive ion channels, the wall-associated receptor-like kinase FERONIA has been implicated in mechanically induced calcium fluxes (Shih et al., 2014). Furthermore, the extracellular maleatin-binding domain of FERONIA has been shown to bind to pectin (Feng et al., 2018). This suggests that other wall-associated receptors, such as THESEUS1 and WAK1, could potentially detect changes in mechanical status of the cell. More recent evidence suggests that MCA1 functions downstream of THESEUS1, potentially mediating responses to changes in mechanical forces (Engelsdorf et al., 2018). THESEUS1 and FERONIA also function as receptors for some of the RAPID ALKalinIZATION FACTOR family of peptides present in the cell wall, which influence cell wall acidification and thereby regulate cell expansion (Haruta et al., 2014; Gonneau et al., 2018).

**Biomechanical feedback regulating directional growth**

The existence of active mechanisms for transducing and sensing mechanical signals in plants permit them to produce an appropriate response. In most cases, the response allows for modulation of mechanical properties of the cell wall, which impacts the growth rates and directions of cells. These changes often involve the existing stresses in cells and tissues, thereby establishing a feedback loop centered on mechanical forces that generate and maintain cell and tissue shapes. Here, I elaborate on such processes that occur in certain cell and tissue types.

**Pavement cells**

Over the past few years, pavement cells have emerged as an excellent model system with which to investigate how mechanical forces are involved in regulating cellular-level morphogenesis. One hypothesis is that the characteristic puzzle-piece shape of pavement cells is generated in order to reduce large open areas of high stresses, by forming undulated regions along the periphery that redistribute stress to indenting domains (Fig. 2A). This permits the cell to achieve a large size, yet keeps the level of stress below a certain threshold (Sapala et al., 2018).

The process of pavement cell shape generation with an emphasis on mechanisms involving anticlinal or periclinal wall has received great attention recently. When tensional forces are applied parallel to the long axis of two adjoining anticlinal walls, which have varying degrees in stiffness, FEM predicts minor bending of the anticlinal walls (Majda et al., 2017). The authors suggest that introducing mechanical feedback into FEM might amplify minor such waving generated by tensional forces. These results have been corroborated by differences in the chemical and mechanical properties of adjacent anticlinal walls (Majda et al., 2017). However, the tension-based model suggests that bending occurs with the stiffer wall being on the concave side of the lobe. This is contrary to growth-restriction models that allow for the formation of undulated regions, in which the stiffer walls are on the convex side (Sampathkumar et al., 2014; Sapala et al., 2018). Anticlinal wall bending has been revisited using a FEM that represents a more realistic three-dimensional cell shape that contains both upper and lower periclinal walls fused along the contour by anticlinal walls (Bidhendi and Geitmann, 2019). The minor bending generated in the tension-based simulations is suppressed when periclinal walls are included. These results indicate that anticlinal wall mechanics alone are insufficient to generate forces to deform cellular contours.

An alternative hypothesis of shape formation proposes that, upon pressurization, anticlinal walls experience anisotropic stresses along a direction perpendicular to the surface of the cell, and compressive stresses parallel to the surface, which result in buckling of the anticlinal wall (Bidhendi et al., 2019) (Fig. 3A). Amplification of minor deformations has been achieved in the model as a consequence of differential material stiffness along the contour of the periclinal wall with stiff domains present on the convex side. These differences in stiffness are initially brought about by the presence of de-methyl-esterified pectin in the future indenting domains (Altartouri et al., 2019; Bidhendi et al., 2019), and occur in the absence of ordered microtubules and CMFs (Belton et al., 2018; Bidhendi et al., 2019; Altartouri et al., 2019). Yet the lack of appropriate *in vivo* tools that permits assessment of compressive forces in cells during growth hinders the evaluation of the FEM.

It is known that microtubules and CMFs are necessary for the formation of pavement cell shapes because disruption of microtubules or cellulose, through drugs or mutations in genes encoding components of microtubules or cellulose synthesis, results in cells with simpler shapes (Fujita et al., 2013; Armour et al., 2015; Altartouri et al., 2019). This suggests that buckling could trigger the initial process of symmetry breaking, followed by pectin-mediated local stiffening at the periclinal wall. Concurrently, cell shape is enhanced by microtubule-dependent cellulose deposition. A more recent model proposes that pectin filament expansion along the anticlinal wall is sufficient to generate undulations (Haas et al., 2020). FEM-based simulations of small sections of anticlinal wall in continuum with upper and lower periclinal walls support this possibility. However, the outer periclinal wall in the FEM, despite being composed of two elements, lack beams of stiff CMF that are far superior in mechanics compared with the pectin, thus might not reflect the actual mechanical status of these walls. As discussed above, the newly deposited anticlinal wall stiffness is not sufficient enough to deform the highly tensed and stiff parental periclinal cell wall. It is therefore unclear whether such a mechanism does indeed contribute to the morphogenesis of pavement cells shape.

**Sepals**

In addition to cell and tissue shape-driven stress, growth of cells in tissues also impose a local mechanical effect. Microtubule orientation in the tip of the sepal is hyper-aligned based on growth-derived signals (Hervieux et al., 2016). It is predicted that a microtubule tension-feedback mechanism at the sepal tip resists the tangential tension, restricting transverse expansion of the tissue and
inhibiting growth at the sepal tips. FEM predicts that either increasing or decreasing the mechanical feedback would result in narrowing or widening the tip domain, respectively. Indeed, the mechanically hyper-responsive mutant of the SPIRAL2 microtubule-associated protein has narrow sepal tips, whereas the katanin mutants (with reduced mechanical feedback) have blunt sepal tips. In addition to differences in mechanical stress occurring over a larger tissue scales, sporadic higher growth rates in cells destined to become trichomes impose a change in the local pattern of stress, which influences the overall sepal shape in the absence of a stress-feedback mechanism. To prevent this, stress feedback mechanisms ensure that these local stresses are buffered by microtubule-mediated cell wall stiffening of these regions, thereby maintaining sepal morphology (Hervieux et al., 2017).

Shoot apical meristem

Cell division

In the SAM, several rules of cell division plane orientation have been proposed, based on the geometry of the cell. The Besson-Dumais hypothesis, based on an extension of the classical Errera’s rule, suggests that cells divide along one of the many shortest paths present in cells, as a single cell has several minimal area configurations (Besson and Dumais, 2011). Examination of relatively isodiametric cells of the SAM has shown a limited number of cells follow the existing cell division rules (Shapiro et al., 2015; Louveaux et al., 2016). However, a mechanical tension-based model of cell division plane orientation closely matches the observed division plane orientation along the boundary domain in the SAM (Louveaux et al., 2016) (Fig. 3B). Cell-ablation experiments have also shown that newly formed cell walls orient parallel to the direction of the stress, independent of cell geometry (Louveaux et al., 2016).

Auxin

The distribution of the plant hormone auxin at the SAM is also under control of mechanical forces. The auxin-efflux carrier PIN1 is recruited to the plasma membrane in a tension-dependent fashion (Nakayama et al., 2012). Growth-driven stresses contribute to the polarization of PIN1, which, upon changes to the mechanical status of cells, redistributes to the plasma membrane that is parallel to the microtubule arrays (Heisler et al., 2010) (Fig. 3B). This process is microtubule independent, yet the presence of auxin is known to promote loosening of the cell wall, causing microtubules to be more randomly organized. This promotes outgrowth of new organs at the

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**Fig. 3. Mechanical-feedback machinery.** (A,B) Schematics of the mechanical-feedback loop that regulates pavement cell morphogenesis (A) and cell division at the shoot apical meristem (B).
SAM (Sassi et al., 2014). Mechanical forces also act independently of auxin to influence robust gene expression of growth regulating transcription factors (Landrein et al., 2015).

Cell wall and microtubules
In addition to auxin, modification to the chemical status of the cell wall polymers, either by application of pectin methyl-esterase or by mutations to xyloglucan biosynthetic enzymes, also impacts microtubule organization at the SAM (Armezzani et al., 2018; Zhao et al., 2019). Perturbation to global amounts of cellulose results in significant reduction of cell wall stiffness at the SAM, yet does not result in cell size defects or tissue level morphological abnormalities, other than the overall reduction in organ size (Sampathkumar et al., 2019). This suggests that, first, an active, cell wall integrity-sensing mechanism could feed back and regulate growth-related processes (such as progression of the cell cycle) to ensure proper functioning of cells and tissues, and second, that cell wall softening is insufficient to promote wall loosening (Wang et al., 2020). A more attractive mechanism in which the cell cycle machinery is directly influenced by the mechanical status of the cell wall is also plausible. Such phenomenon does exist in animal systems in which cell spreading and proliferation are dependent on the mechanics of the extracellular matrix (Wells, 2008). Modifications of microtubule arrays by means of the katanin mutation promotes organ formation when introduced into the pin1 mutant background, which have a SAM without organ outgrowths, suggesting that microtubule organization could also indirectly impact the activity of the cell wall synthesis and the remodeling machinery (Sassi et al., 2014). Indeed, katanin pin1 double mutants highly express cell wall remodeling genes that are typically absent in the pin1 mutants by themselves (Armezzani et al., 2018).

In addition to the above mentioned cell and tissue types, mechanical forces impact polarity markers of the stomatal lineage (Bringmann and Bergmann, 2017). Mechanical forces in hypocotyls impact microtubule organization, with asymmetric pectin deposition enhancing cellulose-driven anisotropic growth (Bou Daher et al., 2018). Tensional tissue-wide stress in hypocotyls gradually shifts the array of microtubules from a transverse to longitudinal orientation; however, this transition is overdriven by application of compressive stresses that maintained the microtubules in a transverse orientation (Robinson and Kühlemeier, 2018). Mechanical stress-based feedback on growth is also essential for vertical proprioception in trees, and defects in such mechanisms result in an imbalance between radial and vertical growth, leading to loss of posture (Alonso-Serra et al., 2020). Together, these studies reveal the existence of a complex feedback machinery involving mechanical stress, the cell wall, auxin and microtubules in plants.

Future perspectives
We have made significant progress in understanding how mechanical forces influence plant development in the past two decades. Yet this is small when compared with the progress made in the field of hormonal or developmental gene regulatory networks. FEMs have helped in deciphering mechanical aspects of morphogenesis; however, FEM is not without its limitations (Box 3). Another important question is are the principles of the mechanical-feedback loops discovered in the examples discussed above conserved in other cells and organs? This has yet to be investigated in detail. Limitations of in vivo measurements of stress and strain also hamper our attempts to evaluate the predictions obtained in mathematical models. Despite microtubules acting as a good proxy for stress distribution, development of Foerster resonance energy transfer-based genetic sensors would allow visualization of these parameters as cells grow and develop. Mechanical forces are also transduced and sensed at different time and spatial length scales, depending on the nature of the material properties, as well as the dimensions of cell and tissues. Identifying molecular components that function at these different scales would help us exploit the importance of mechanics in regulating cellular functions.

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