The Role of Mechanical Forces in Plant Morphogenesis

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Abstract

The shape of an organism relies on a complex network of genetic regulations and on the homeostasis and distribution of growth factors. In parallel to the molecular control of growth, shape changes also involve major changes in structure, which by definition depend on the laws of mechanics. Thus, to understand morphogenesis, scientists have turned to interdisciplinary approaches associating biology and physics to investigate the contribution of mechanical forces in morphogenesis, sometimes re-examining theoretical concepts that were laid out by early physiologists. Major advances in the field have notably been possible thanks to the development of computer simulations and live quantitative imaging protocols in recent years. Here, we present the mechanical basis of shape changes in plants, focusing our discussion on undifferentiated tissues. How can growth be translated into a quantified geometrical output? What is the mechanical basis of cell and tissue growth? What is the contribution of mechanical forces in patterning?

Contents

INTRODUCTION

In plant tissues, unlike in animal tissues, shape changes are usually achieved without cell migration and without cell removal. Thus, mechanistically, shaping a tissue requires the coordination of three core processes at the cellular level: the expansion of the contact area between cells, the alteration of the shape of a cell, and the creation of a new wall within a cell. The differential control of these processes in specific domains, via factors such as master regulatory genes, creates the changes necessary to generate the final design of the tissue or organ. Because a growing structure is a physical object, understanding shape changes, a geometrical output, requires the full assessment of its mechanical status, beyond the molecular control of growth (13, 54, 67).

Here, we will not discuss the molecular aspects behind shape changes. Reviews on the cell wall composition, cytoskeleton, and impact of molecular signals and gene network on growth patterns can be found elsewhere (e.g., 8, 14, 18, 27, 31, 46, 159). We focus on the role of mechanical forces in plant morphogenesis. Furthermore, we do not consider the role of

external forces but concentrate on intrinsic mechanical signals caused by growth. Examples are taken from different plant tissues, but as meristematic tissues contain stem cells and coordinate organ growth, their contribution to plant architecture is given more attention. Data from the other kingdoms are also included whenever helpful for understanding the mechanics of growth in plants.

We first present current protocols to quantify the geometrical changes during growth, thus underlining the need for quantitative imaging to formulate and test plausible hypotheses. We next analyze how these geometrical changes rely on the mechanics of walls, cells, and tissues. Finally, we discuss how these mechanical properties and forces can help control morphogenesis, thus acting as mechanical signals.

THE GEOMETRY OF GROWTH

Understanding morphogenesis implies measuring changes in tissue or organ shape over time and linking them to the dynamics of their component parts. This growth can be described in purely geometrical terms, by assessing the precise nature of the irreversible deformations that occur within the tissue over time.

Growth fields can be quantified using three primary metrics: growth rate, growth anisotropy, and growth direction. Growth rate is a measure of change in size over time, often normalized against initial size (i.e., relative growth rate). Growth anisotropy is a measure of the similarities or differences between growth rates along the various axes; similar rates imply isotropic growth and dissimilar rates imply anisotropic growth. Growth direction is simply the direction in which anisotropic growth occurs. Tissues undergo changes through either more or less anisotropic growth of individual cells or through coordinated combinations of cell growth in different subdomains (8). Some recent review articles cover these concepts in an exhaustive manner (13, 27, 54). However, less attention has been given to reviewing the tools that aid in observing and measuring growth
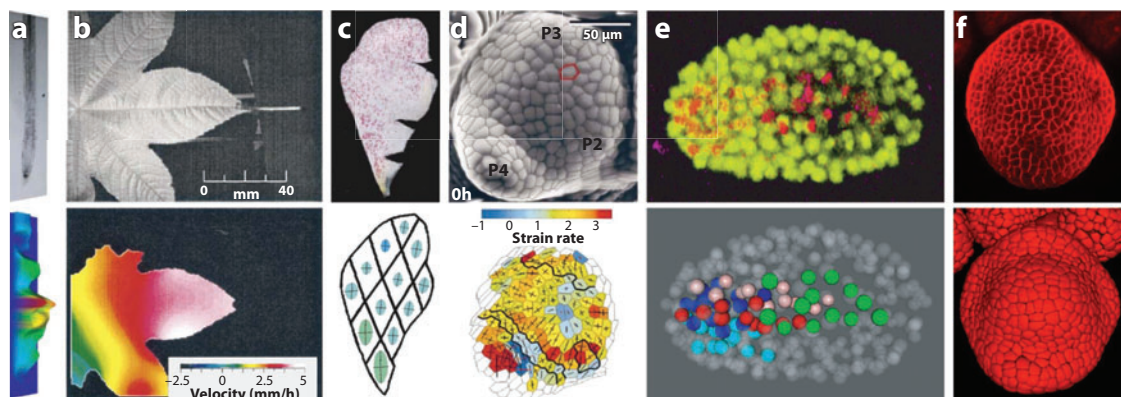
Figure 1

Accessing growth and geometry from time-course experiments. Upper panels show images of the data gathered in the various studies, and lower panels illustrate analyses of growth or geometry. (*a*) Photograph of a growing root in *Arabidopsis thaliana* and a plot of its growth field coded by color and height. Adapted from Reference 24. (*b*) Photograph of a *Ricinus communis* leaf used to generate a velocity field. Here, color is based on the X component of the velocity field, representing the proximo-distal expansion. Adapted from Reference 142. (*c*) Photograph of an *Antirrhinum* petal bearing clones of cells (*darker spots*). Growth is represented on an average petal shape as deformation ellipses. Adapted from Reference 138. (*d*) A scanning electron micrograph of a replica of an *Anagallis arvensis* vegetative meristem. Cellular growth anisotropy is represented by segments, whereas growth rate is shown in color. Adapted from Reference 88. (*e*) A three-dimensional (3D) projection of a 350-cell-stage *Caenorhabditis elegans* embryo, in which all nuclei are marked in yellow, with a specific gene reporter visualized in red. A 3D model of the same embryo, with lineage data automatically extracted. Adapted from Reference 103. (*f*) A 3D projection of a young *Arabidopsis* flower bud, with cell outlines stained in red. A 3D model of the same bud with cell geometries and lineages automatically extracted. Adapted from Reference 50.

fields and their roles in tissue design. This section therefore focuses on the various ways in which the problem of analyzing the emerging geometry of developing organs has been tackled over the years (**Figure 1**).

Initial studies focused on understanding growth at the tissue level. **Figure 1*a*–*c*** shows photographs taken at defined intervals during tissue growth, using either histological landmarks on the surface of the organ or marked cell clones to track the fate of particular points over time (4, 47, 123, 138, 142, 156). Growth rates and growth anisotropies were determined in different parts of roots, leaves, or petals. Early work on roots mainly focused on the primary root axis (i.e., on one dimension) and showed that growth is generated principally through two mechanisms in different regions of the root. Thus, although one zone may display cell division (by increasing either the division rate or

the number of dividing cells), others may display cell elongation (48). Rolland-Lagan and colleagues (138) also used computer-based dynamic growth simulations to integrate and analyze the growth parameters observed during *Antirrhinum majus* petal development. They modeled the tissue as a grid, whose regions are linked by springs with resting lengths corresponding to that of the mature organ. They then diminished these lengths so as to correctly align the tissue grid between all time points, based on the clonal tracking data. Thus, in effect, the mature tissue is shrunk from the originally modeled grid until it reaches the initial observed state. By modifying the parameters within their model, they were thus able to investigate the relative contributions of the different growth parameters. Surprisingly, they found that the final shape of the petal depends more on the principal growth axis (i.e., growth direction)

than on the local differences in growth properties (i.e., rates and anisotropies). A limitation in these studies is that little access is provided to some important cellular properties (e.g., cell division rates, planes, anisotropies, etc.). Because this is an issue associated with the methodology used, it cannot be overcome by simply increasing the density of the landmarks used (156).

An alternate protocol was designed to measure growth at cellular resolution (40, 88, 161, 162) (**Figure 1d**). In this nondestructive imprint method, a (negative) mold is first made of the desired tissue using a pliable dental polymer. A (positive) cast is then prepared by filling the mold with a hard resin, which is imaged using scanning electron microscopy. This generates very high-quality images of the surface of the growing tissue. Dumais & Kwiatkowska (40) advanced this method by imaging each replica from two different angles and using stereoscopy to reconstruct a 3-dimensional (3D) virtual surface of the apical tip of the *Anagallis arvensis* shoot, to calculate the curvature of each cell. By mapping cell correspondences at different times during shoot growth, they then assessed the fate, as well as the growth rate and the growth anisotropy, of each cell. They also calculated the cell division rates and 3D deformation of the surface through time. This provides convincing evidence that specific regions on the surface of the shoot apex (such as the center, periphery, or boundary between organs) have cells with particular properties or geometries (88). One unavoidable limitation of this procedure is that it cannot be used to study the growth of internal cells.

Methods developed in the animal field permit the examination of growth in three dimensions by tracking nuclei through time using standard confocal laser-scanning microscopy (CLSM) (98, 103, 148) (**Figure 1e**). These data can be acquired to approximate growth fields and cell–cell neighborhood graphs in entire tissues, while simultaneously visualizing cell fates, via the use of fluorescently tagged reporter constructs. A technique called digital scanned laser light sheet microscopy (DSLM) was recently

created, which presents several advantages over CLSM, including high scan speeds, quantitative imaging, and images of very high quality (80). Imaging fluorescently labeled nuclei every 60–90 seconds at high resolution over approximately 24 hours, Keller and colleagues (80) followed the development of an entire zebrafish embryo (a volume of approximately 1 mm^3) through tens of rounds of cell division, until each embryo was made up of several thousand cells. Using the 3D patterns of cell divisions and migrations, they were able to detect the dorso-ventral axis well before any morphological manifestation of it.

Because such information is crucial to understanding how a tissue is restructured during morphogenesis, this represents a major advantage. A drawback, however, is that nuclear tracking provides no useful information on cell shape and size. Because the cell wall in plants plays a crucial role in the dynamics of tissue growth, a thorough description of morphogenesis necessarily requires imaging methods able to gather data on those walls. Thus, protocols have been developed to permit the observation of cell shapes in living tissues (57, 133). A more recent improvement on this technique allows a digital reconstruction of cell shapes in an entire growing organ and, furthermore, a semiautomated lineage tracking of all those cells (50) (**Figure 1f**). Fernandez and colleagues (50) show that as the *Arabidopsis thaliana* flower grows, the cells in the flower go through cycles of division and expansion that are more or less similar from one region to another. However, during a precise development window, the cells in the region destined to become the pedicel (the base of the flower) alter their behavior and grow through cell expansion rather than through an increase in division rate. More generally, this method allows for a thorough investigation of volumes, growth rates, and anisoptropies for all cells in a growing tissue. An obvious limitation is that the technique does not provide access to lower-level data, e.g., at subcellular resolution.

Thus, over the years, growth analyses have gone from one dimension at the tissue level to

three dimensions and with cellular resolution. Understanding how this growth is established and regulated can be addressed on several levels. The genetic regulation of growth can be addressed (via the analyses of cell fate markers or mutants) with data that do not have cellular resolution. However, deciphering the mechanisms by which genes bring about geometrical changes requires knowledge about the mechanical properties of cells in four dimensions.
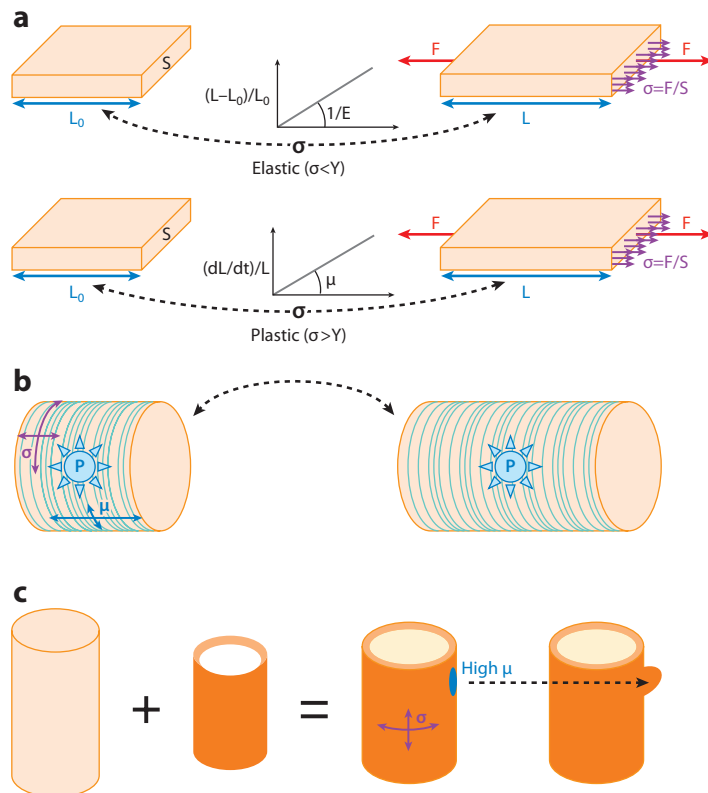
THE MECHANICS OF GROWTH

In plants, the main structural element of the cell is its exoskeleton, the cell wall, and the modifications in its mechanical properties are the main determinant of shape changes. How are these modifications achieved? What are the mechanics behind growth patterns?

Mechanics of Walls, Cells, and Tissues

Mechanics is all about how objects change in shape when subjected to physical forces. Solids can be elastic, recovering their initial shape when released from external forces, or plastic, failing to recover their initial shape when released and instead slowly, irreversibly elongating (known as creeping) while the forces are applied. Examples are a piece of rubber, which remains elastic as long as it does not tear, and a piece of plastic bag, which deforms irreversibly when subjected to large forces. As discussed in the next section, growth is related to plasticity of the cell wall.

As an example of a simple elastic structure, consider a body in the shape of a plate of length L_0 and cross-section area S (**Figure 2a**). In order to deform the plate and increase its length to a value L, it must be put in tension by applying two opposite forces of magnitude F at its ends. If the plate is elastic, the relative increase in length, or strain, $(L - L_0)/L_0$, is proportional to the force F that is applied to the plate. As the impact of F on the strain also depends on the thickness and width of the plate (through S, the surface area of its cross-section),

Figure 2

The mechanics of growth. (*a*) The mechanics of the wall. A piece of cell wall of length L and cross-section of area S is subjected to forces of magnitude F. Stress σ is the force per unit area exerted on the cross-section. If the stress σ is smaller than the yield threshold Y, the wall remains elastic and recovers its initial shape if released from forces. The strain $(L - L_0)/L_0$ is proportional to stress; the modulus E characterizes the stiffness of the material. If the stress is larger than the yield threshold, the wall creeps irreversibly. The rate of strain (dL/dt)/L is proportional to stress, μ being the extensibility. Growth is the combination of wall yielding and incorporation of new materials in the wall. (*b*) Anisotropic growth of a cell. A typical cylindrical cell, with walls put in tension by turgor pressure P. Stress in the circumferential direction is twice as large as longitudinal stress. If cellulose microfibrils are circumferential, then extensibility is much higher in the axial direction. As a result, growth is mainly longitudinal. (*c*) From tension in the epidermis to morphogenesis. If extensibility of the epidermis is smaller than that of internal tissue, then an isolated epidermis would retract (the retraction is emphasized for clarity), implying that the epidermis is in tension. A region in the epidermis with an enhanced extensibility would tend to form a bump.

mechanical stress has been introduced and defined as the force per unit area σ = F/S and is the relevant quantity to describe the mechanical state of a body. For an elastic body, the relation between strain and stress can be

Strain: the measure of the deformation (in length, surface, or volume) of a body from an initial state to its final state

Stress: the measure of the force per unit area of a surface upon which forces act

Elastic modulus: the ratio between stress and strain, which provides a measure of the stiffness of a given material

written as $(L - L_0)/L_0 = \sigma/E$, E being the elastic modulus of the material (**Figure 2a**). Although we simplify the presentation by restricting our attention to shape changes along one direction, we note that the concepts of strain and stress can be generalized to 3D shape changes (13).

The elastic modulus (E) has units of force per area (Newtons per square meter, N/m^2, i.e., Pascals, Pa); the higher the E, the stiffer the material. For instance, cellulose (modulus of approximately 100 GPa) is much stiffer than matrix polymers, such as lignin (2 GPa), hemicellulose (40 MPa), or pectins (10–200 MPa) (17, 107, 172). Typical moduli for the cell wall range from 10 MPa to 10 GPa (12, 79) and vary according to the composition (83). However, a single modulus is not sufficient to describe the wall properties because of the anisotropy imposed by cellulose microfibrils: The modulus can be five times larger in the main direction of microfibrils than in the perpendicular direction (82). At the multicellular level, the values of moduli are also widely distributed following tissue types (107). Methods used to measure moduli are chosen according to the size of the samples and to their expected stiffness (53). However, the multiscale nature of the mechanics of plants (walls, cells, tissues, organs) makes the interpretation of measurements quite subtle (15). Modeling approaches using a refined representation of the structure, such as finite element models (11, 66), can help determine mechanical properties of the tissue.

Plastic (irreversible) properties are more difficult to measure because they involve dependency on time. For instance, a viscoplastic plate (length L and cross-area S) remains elastic if subjected to a tensional stress F/S smaller than a threshold Y, whereas the plate irreversibly increases in length if the stress $\sigma = F/S$ is larger than Y, according to the law $(dL/dt)/L = \mu(\sigma - Y)$, where dL/dt is the time-derivative of L, and μ is the extensibility of the material. As discussed below, this law makes the link between a plastic behavior and growth, which are both irreversible. Extensibility μ has units of area per force and per time, $m^2/N/s$; the

larger the μ, the more rapidly it yields for a given tension. In the absence of measurements, it is natural to make the hypothesis that extensibility varies inversely with stiffness. In other words, a stiff wall would creep at a slower rate than a soft wall. Similarly, we can expect extensibility to be lower in the direction of cellulose microfibrils (**Figure 2b**)

Even without external stimuli, plants are under tremendous forces of osmotic origin. Turgor pressure is a force per unit surface applied by the protoplast on the cell wall and is generally in the range of 0.1–1 MPa (53). This is indeed much larger than, for instance, the pressure in an inflated rubber balloon (5 kPa). Turgor pressure can be measured directly by using a microcapillary to pierce the wall (62) or indirectly by changing external osmolarity or by mechanical manipulation of cells (53, 96, 158). Turgor inflates a cell, much like a rubber balloon, and puts the walls under tension. If the walls are plastic and this tensional stress is large enough, then they creep, thus contributing to growth.

Cell Level

Growth of a single cell is generally formalized using Lockhart's equation (97, 132). It states that the volumetric rate of growth (dV/dt)/V is proportional to turgor pressure P in excess of a growth threshold π, $(dV/dt)/V = f(P-\pi)$, f being the cell extensibility. Note that we focus on the mechanical aspects and discard water potential, which is discussed elsewhere (54, 143). Lockhart's equation is supported by measurements on single algal cells, such as the internodes of *Nitella* (62) or *Chara* (127) [for a review, see, e.g., (54, 144)]. This equation describes the irreversible increase in volume, and it can also be extended to account for the reversible elastic behavior of the cell (54, 127, 128).

Lockhart's equation is formally similar to the viscoplastic law, which would describe the yield of a single wall. As walls are put into tension by turgor, they yield when the tension is sufficiently large. Thus, the pressure

threshold π must be related to the yield threshold Y of the wall, whereas f should be given by the thickness of the wall and its extensibility μ. Lockhart's equation appears to be a way to describe wall plasticity at the cell level. However, growth differs from plasticity in that new materials are continuously added to the wall. For instance, incorporation of pectins in the wall requires turgor to be larger than the threshold for growth (125, 126).

We now focus on the control of growth through action on cell walls, which may involve the control of thickness, turgor pressure, or extensibility. Thickness does not seem to be correlated with growth (35, 134) but may be regulated independently: High tension of the plasma membrane is known to favor exocytosis (2); higher growth rate may increase this tension and thus enhance the delivery of materials to the walls, helping to compensate for the thinning induced by wall creep. There are indirect indications that turgor pressure is regulated in the context of the shoot apical meristem (29) or of the tip growth of pollen tubes (170, 171), but such a regulation seems to be excluded as causing the oscillatory growth of pollen tubes (164).

It is generally thought that the regulation of wall extensibility is the primary factor in the control of growth (31). Several classes of molecules are involved in wall loosening, i.e., wall extensibility (31). As an illustration, we consider here the large classes of expansins and pectin methylesterases (PMEs). The application or overexpression of expansins increases growth (52, 101, 122), whereas their inactivation reduces growth (25, 26). Note that expansins are activated at low pH (acid growth), which adds another layer of control, through proton pump activation, on wall loosening (31). PMEs induce the demethylesterification of pectins (119), uncovering carboxyl groups that can crosslink through calcium bridges, stiffening the matrix (36, 76). Demethylesterification of pectins is also a prerequisite for the activity of pectate lyases, but leading instead to wall softening by degradation. In the absence of Ca^{2+}, demethylesterification is also thought to soften the matrix and may allow for a higher growth rate in primordia than in the shoot apical meristem (117). Finally, although the action of these enzymes is thought to control extensibility, they may also have an effect on the yield threshold.

As turgor is nondirectional, further control is required to generate the elongated shape of many cell types. Isolated cells such as the internodes of *Nitella* or *Chara*, pollen tubes, or root hairs can be modeled as shells inflated by turgor, yielding a tensional stress in the walls (12, 20, 22, 41, 56). One notable result is that stress along the circumferential direction of the cell is twice as large as the stress in the axial direction (22) (**Figure 2b**). As a consequence, a higher growth rate is expected in the circumferential direction, in contrast with observations. Two strategies allow plants to circumvent this difficulty: restricting growth to a localized region or reinforcing cell walls anisotropically. In pollen tubes or root hairs, growth is restricted to the cell tip and is mediated through the local softening of the wall at the tip (54), as supported by indirect mechanical measurement of wall properties (169). Local regulation by PMEs seems to be important in this local softening (76).

In many cases, the cytoskeleton is crucial in anisotropic growth. During the early development of metazoans, elongation can be driven by the anisotropic contraction of cortical actomyosin, such as that which occurs in *Caenorhabditis elegans* (99) and *Drosophila melanogaster* (131). In plants, cortical microtubules (CMTs) play a similar role: Their depolymerization leads to isotropic growth (29, 57, 61, 66). However, in contrast to actomyosin in animals, CMTs in plants have a negligible mechanical role as this depolymerization has no immediate effect on shape. In fact, the role of CMTs is indirect: They generally guide the deposition of cellulose microfibrils on the cell wall (115), thus leading to the reinforcement of cell walls along a well-defined direction, and consequently greatly reduce growth in this direction (**Figure 2b**); this occurs often along the circumference of the outer walls of the root and the stem (8, 31, 149). Conversely, tissues with isotropic growth, such as the epidermis of young hypocotyls and the summit of the shoot

CMT: cortical microtubule

apical meristem (SAM), tend to exhibit a continuous reorientation of CMTs (23, 66).

To sum up, plant cells have the ability to act autonomously on the walls to control the rate of growth and its anisotropy. This raises the question of how conflicting instructions between neighboring cells are resolved, which brings us to the tissue and organ level.

SAM: shoot apical meristem

Tissue and Organ Level

The separation between epidermis and internal tissues is an essential feature of higher plants (140). As the epidermis acts as a barrier, it may seem reasonable to think that it is stiffer than internal tissues. Indeed, epidermal walls can be 5 to 10 times thicker than internal walls. Following the same argument as above, the extensibility of epidermal walls is expected to be lower. As a consequence, an isolated epidermis would have a lower growth rate (**Figure 2c**), implying that the epidermis is in tension in planta (87). Indeed, epidermal peels tend to become shorter after excision (71, 121), and cuts in the epidermis tend to remain open (42, 135). The restoration of dwarf mutants by the induction of the growth-promoting brassinosteroid activity in only the epidermal layer (also called L1) seems to support the view that the epidermis limits growth (140). Nevertheless, the relative roles of the epidermis and inner tissues still need further clarification. For instance, in the SAM, expansin activity in the L1 is sufficient for a bump (52), but its induction in all layers is required for the development of an organ (122), whereas the demethylesterification of pectins occurs in the L2 and L3 inner layers (117). The relative roles of epidermal and inner tissues will be further discussed below.

It appears that, when considering tissue and organ levels, intuitive arguments become more and more difficult. On the one hand, the measurement of local mechanical properties (53, 107) would be very useful. On the other hand, theoretical models of cells and tissues can help explore the consequences of various hypotheses on cell and wall behavior. A first class of models keeps track of the cellular resolution and represents all walls (or, sometimes, interactions between cells). In two dimensions (flat or curved surfaces), spring models use beam elements for which values of stiffness and extensibility are defined, which can be viewed as the simplest models for a cell wall (129). Spring models have been used as a growing template to model signaling in space (44, 77) or to test morphogenetic rules (43). Their use has facilitated investigation of hypotheses on the mechanical behavior of cells, e.g., the difference in stiffness between procambial and ground cells in leaves (28), the orientation of microtubules in the SAM (66), or turgor regulation in the SAM (29). Potts models (58) can have a finer resolution and were used as a growing template for a subcellular model of auxin transport in the root (65).

Continuous mechanical models, which do not track individual cells and describe average behaviors over many cells, form a second class and can help investigate questions at the tissue level (150). They have been used to show that the hypothesis of compressive stress in the epidermis can account for phyllotaxis (60, 145). They also helped test the hypothesis of stress-induced differentiation of ground cells into procambial cells (30, 32, 89). Finally, continuous models of thin organs (3, 37, 45) were used to investigate how simple growth profiles can generate complex, 3D wrinkled leaf shapes (105, 114) or dome-like leaf shapes (160). More generally, cell-based and continuous models are useful in investigating how the coordination of cell or tissue identities is at the basis of morphogenesis.

MECHANICS AS AN INSTRUCTING SIGNAL

All developing organisms experience internal mechanical stress. The resulting diagram of forces (34) may act as a coordinating supracellular signal (9, 63, 95). Several studies, mostly on animal systems, have uncovered a role of mechanics in controlling growth patterns. Several putative mechanosensors have also been identified. We discuss these issues in the context of plant development.

Growth Direction

An important parameter associated with me-
chanical forces is their directionality. Biolog-
ical tissues use such directional information to
control their growth pattern.

A classical example illustrating the impact of
mechanical forces on the architecture of a tissue
is Wolff's law, which states that the bone con-
stantly adapts to its mechanical environment
(165). Typically, in a human femur, the net-
work of trabeculae constantly remodels itself in
order to resist changing stress directions and
intensities during growth, aging, or locomo-
tion, in particular by favoring one major orien-
tation parallel to maximal (compressive) stress
directions and by inducing or repressing local
thickenings (116). To further understand the
mechanisms behind Wolff's law, 2D and 3D fi-
nite element modeling were developed; results
from the simulations are consistent with com-
plex bone transformations arising from a local
cellular response to the loading environment
(153, 154).

At the cellular level, and as in Wolff's law,
the plant cell wall has been predicted to con-
stantly remodel itself in order to provide re-
sistance to the main (tensile) stress direction
(64, 163). Given that the CMTs control the
orientation of cellulose deposition, and thus
the anisotropic mechanical properties of the
cell wall, imaging the dynamics of the CMTs
can indirectly reflect the remodeling of the
cell wall. A careful analysis of the orientation
of the CMTs in the SAM revealed that they
indeed align along the main stress direction
(**Figure 3**).

When the stress pattern was modified
through cell ablation or tissue compression,
CMTs reoriented parallel to the new stress di-
rection. Mass spring modeling further showed,
as in bone, that a simple local response to stress
is sufficient to explain the complex morphogen-
esis occurring at the SAM (66). Interestingly,
an analogous, although actomyosin-based, me-
chanical feedback seems to occur in animal cells.
In particular, it has been shown in *Drosophila*
that the local increase of tension at the plasma
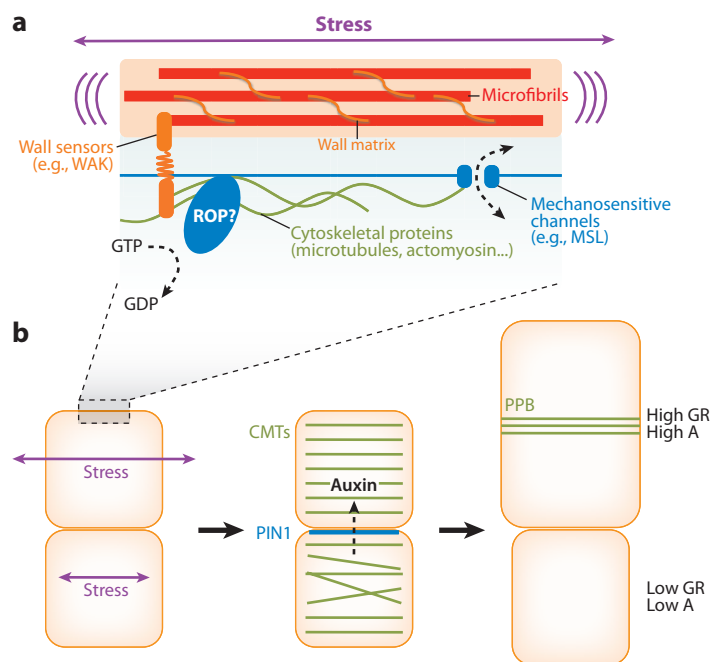
Figure 3

Sensing mechanical stress in plant cells. (*a*) Mechanical stress (*purple*) may be
transmitted via the cellulose microfibrils (*red*) and the wall matrix (*orange*) to
the cytoplasm via cell wall sensors like the Wall associated Kinases (WAK),
Lectin receptor kinases (LecRK), Proline-rich extensin-like receptor kinases
(PERK), leucine-rich extensin proteins (LRX), Arabinogalactan proteins
(AGP), and Theseus 1 (THE1). Other proteins link the wall to the plasma
membrane (*not shown*). Mechanosensitive channels like the MscL-like proteins
or putative membrane-associated mechanosensitive proteins like MCA1 are
likely to be involved in this response too. Cytoskeletal proteins may either
contribute to the transduction of the mechanical stress in the cytoplasm or
sense mechanical deformations, as in contractile cells. Mechanical stress may
stimulate nucleotide exchange (GTP/GDP) on the Rho of Plants (ROP)
proteins, even though such an effect has not been documented in plants to date
(73). The cascade may reach the nucleus and modulate the expression of
mechanosensitive genes like the *TOUCH* genes (94). (*b*) Assuming that cells are
under a dominant directional stress, cortical microtubules (CMTs; *green*) will
orient parallel to the main stress orientation, resist it, and thus lead to
anisotropic growth perpendicular to the orientation of the CMTs. Stress will
therefore also impact on the orientation of the preprophase band and that of
the next division plane (66). A scenario is shown (*bottom*) in which two cells are
under stress with similar orientation but different intensities. Cells with the
highest stress (*purple arrow*) are predicted to display enhanced orientation of
CMTs and anisotropy (A). PIN1 (*blue*) is predicted to localize on the
membrane adjacent to the most stressed wall, thus leading to local
accumulation of auxin and to differential growth rates (GR) (70).

membrane stabilizes myosin II at the cell cortex, thus inducing the formation of actomyosin cables and impacting cell elongation and tissue morphogenesis (51, 93, 124).

Cell Division Patterns

Because we know that mechanical forces can control growth patterns, it seems logical to investigate whether cell division, a key parameter of growth, is under mechanical control.

In *Drosophila*, when the wing imaginal disk reaches its final size, cell division stops at the periphery of the disk, and later the rest of the 50,000 cells stop dividing (71). Because the cell cycle arrest occurs synchronously and the distribution and activity of morphogens (e.g., decapentaplegic) remain constant during disk growth, it is possible that the signal inducing the cell cycle arrest is biophysical in nature. In this scenario, the growth arrest at the disk boundary would generate compressive forces that can be transmitted instantaneously and over long distances to the growing cell population (71, 146). Although computer simulations demonstrate that this scenario is plausible, experimental proof of such a hypothesis remains to be shown.

The orientation of the plane of cell division itself may be controlled by mechanical forces. Experiments conducted in human HeLa cells show that the orientation of the mitotic spindle can be influenced by cell adhesion that locally generates mechanical forces at the cell cortex (151). Similarly, in plants, the crease appearing in the boundary domain between the emerging organ and the meristem is predicted to experience a strong orthoradial stress. Interestingly, 90% of the cells in this domain display a division plane parallel to the main stress direction (66). This supports the idea that mechanical stress, via the impact on the CMTs and the preprophase band (a group of microtubules at the equator of the cell predicting the position of the next phragmoplast), can impact on the cell division plane in plants. Several proteins have been shown to control the cell division plane orientation (102, 147, 155). The interplay between

mechanical stress and these molecular effectors will certainly provide interesting findings in the near future.

Cell Fate

In addition to controlling the geometrical aspects of growth, such as growth direction or cell division patterns, mechanical forces have also been involved in determining cell identity, thus providing positional information. Examples of studies supporting this view include the establishment of left–right asymmetry in mammal embryos via a leftward flow of extraembryonic fluid that is generated by motile cilia (108), and the separation between anterior and posterior cell populations in the *Drosophila* wing imaginal disk in a site of increased tension that could prevent mixing of cell populations (1, 91).

In plants, accumulating evidence points to the hormone auxin as the predominant signal triggering positional information. Interestingly, auxin distribution, and its impact on morphogenesis, has also been correlated with patterns of mechanical stress. For instance, auxin distribution is thought to control the formation of the vein network in tissues (139). The similarities between cracks in drying gels and leaf venation patterns suggest that procambial fate could also be determined by mechanical stress (32). Computer simulations and in vivo analysis of growth patterns further support a role of mechanical forces in driving the reorganization of the vein pattern during growth, in parallel to auxin (28, 89).

In the SAM, in parallel to auxin-based patterning mechanisms (136), the buckling model states that compressive forces can induce local bending events that impact the spatialization of organ initiation events (60). Supporting this mechanical model is the fact that compressing a *Graptopetalum* meristem can induce ectopic leaves (63). Similarly, in the sunflower capitulum, the combination of mechanical modeling and cuts made through the epidermis indicates that the epidermis is under compression in the saddle-shaped region where primordia start to be morphologically apparent (42). Continuous

mechanical models show that such a compressive stress can generate phyllotactic-like bump patterns (145). This buckling hypothesis probably requires some adjustments, as cuts made in a tomato SAM suggest that the epidermis is under tension (135), whereas primordia are specified by auxin accumulation long before they are morphologically defined (85). Furthermore, the local application or induction of expansin (52, 122) and PME (117) triggers a bump, suggesting that the epidermis is under tension there (**Figure 2c**). Both processes could actually act cooperatively, thus accounting for the observed stability of the organ initiation pattern (68, 106). Using different micromechanical and pharmacological approaches, it has notably been shown that the auxin efflux carrier PIN1 preferentially localizes to the membrane next to the most stretched wall. A model has been proposed in which auxin would locally soften the cell wall and thus induce a local tension in the adjacent wall, which would locally recruit PIN1 to the membrane. In such a framework, the local response of PIN1 to mechanical stress would allow the formation of supracellular PIN1 patterns, and thus the generation of auxin peaks, cell reprogramming, and organ emergence at well-defined positions in the SAM (70) (**Figure 3b**). Interestingly, lateral root initiation can also be induced by mechanical manipulation, and this can be correlated to the relocalization of PIN1 protein in root protoxylem cells (39).

Gene Expression

Consistent with the impact of mechanical forces on cell fate, the expression of certain genes has been shown to be under mechanical control. For instance, Nowlan and colleagues (109) modified the pattern of forces with a neuromuscular blocking agent and observed an impact on the expression of Collagen X and Indian hedgehog, two genes that regulate the developing bone in the avian embryo limb. They confirmed that the expression of these proteins colocalizes with the predicted pattern of stress, computed using the finite

element method. In the *Drosophila* embryo, the expression of the *TWIST* gene occurs during gastrulation, i.e., a time when large-scale tissue movements occur, and in a domain under strong compression (49). Forces applied artificially with a microvice, with magnetic beads, or via cell ablation further confirmed the induction of *TWIST* expression when the local pattern of stress was modified (38, 49).

Mechanical forces have also been shown to influence gene expression in plants. In particular, several genes are rapidly induced by touch in plants (94). A number of these so-called *TOUCH* genes encode wall proteins that can make the stem thicker and shorter, and thus more resistant to external mechanical constraints, like the wind. It must be noted here that the expression of the *TOUCH* genes is associated with elastic deformation generated by discontinuous stimuli. Growth, which is plastic in essence, may use different molecular effectors to respond to internal constraints. Nevertheless, some of these genes are likely to be relevant markers of modified stress patterns. In woody plants, ZFP2, a mechanosensitive transcription factor, has been studied in more detail. In particular, mechanical stress rapidly induces the expression of ZFP2 at a higher level than other stresses, and after bending a stem, ZFP2 is expressed only in the mechanically strained tissues (92, 100). Interestingly, the accumulation of ZFP2 mRNA is linearly correlated to the mechanical strain, strongly suggesting a fine-tuned and supracellular regulation of this gene in response to mechanical forces (33).

Sensing Mechanical Forces

If mechanical forces control gene expression, growth, and supracellular patterns, how are they sensed? At the molecular level, mechanical forces can modify the conformation of proteins. This can either uncover an active site and/or change the neighborhood between proteins in a complex. Many proteins have been shown to deform in response to mechanical forces (110, 157), and changes in the conformation of proteins under tension have been monitored by

atomic force microscopy (111). Mechanosensors can therefore be defined as proteins that deform or unfold in response to a mechanical force and thus transduce the stress into a deformation (e.g., a protein conformation change) that can be interpreted as a biochemical output. In addition, the response to forces could be better amplified if these deformable proteins were directly connected to a large element that can easily transmit the forces (112). Typically, in plants and in animals, the extracellular matrix and the cytoskeleton could very well fulfill this task.

Many mechanosensors have been identified in the past 15 years in animal systems (81, 110, 111, 137, 152, 157). In contrast, this field is just emerging in plants (**Figure 3a**). Here, we have selected findings from the other kingdoms that could shed some light on putative analogous pathways in plants.

Because the forces in the tissue first act at the interface between neighboring cells, the primary site of force sensing is likely to be at the extracellular matrix (ECM). In animals, the focal adhesion site is arguably one of the best studied examples of a mechanoresponsive site (10). The stretching of fibronectin in the ECM can expose binding sites for the heterodimeric transmembrane receptor of the integrin family (5, 6, 84), which after binding leads to a modification of the conformation of integrin, which in turn allows its interaction with intracellular partners such as talin (55, 74). Importantly, integrins link the ECM to the cytoskeleton via a complex network of proteins, forming a submembrane plaque. The link with actin is notably established by talin but also by α-actinin, filamin, and tensin (10). Last, many of the focal adhesion plaque proteins exist in two conformations (inactive and active), and for some of them, change in conformation is mechanically controlled. For instance, it has been shown that force-dependent change in the conformation of α-catenins allows them to recruit vinculin, an actin-binding protein, to sites of cell adhesion (168). Vinculin itself has actually been recently used to build a FRET tension sensor that can reveal the transmission of forces in focal adhesion sites (59). Mechanical stress may even be transmitted as far as the nuclear envelope: Mutations in the lamin C gene not only alter the mechanical properties of the nuclear lamina but also impact on stretch-induced gene expression (90). The expression of integrin itself can be controlled by mechanical stress: It has recently been shown that the expression of the α-subunit of an integrin heterodimer can be induced in cells attached to a collagen gel and inhibited when cells are grown in floating gels (21).

Plant genomes do not have obvious integrin homologs. Nevertheless, because the Arg-Gly-Asp (RGD) motif is present in the protein ligands of several integrins, RGD peptides have been applied to plant cells and have been shown to disrupt the link between the cell wall and the plasma membrane (also known as Hechtian strands) (75). Because Hechtian strands also contain actin and microtubules, they represent an ideal site for force transduction from the cell wall to the plasma membrane and the cytoplasm. Interestingly, several putative cell wall sensors can bind the RGD sequence (73).

Stretch-activated channels represent another main point of entry for mechanical signals. For instance, the mechanosensitive channel blocker gadolinium is able to prevent the rapid and transient increase of intracellular calcium following mechanical stimuli in animal cells, and notably this response has been involved in bone formation (16, 130). The mechanoresponse of the bacterial mechanosensitive channel of large conductance (MscL) has been studied in the most detail. If the membrane is stretched, the balance of forces associated with surface tension is altered, and the minimal energy for protein conformation can change. It has been shown that the physical distortion of the membrane opens the pentameric MscL channel. Models predict that conformational changes involving the two α-helices of each MscL monomer cause the pore to open (86, 120, 157).

Ten MscS-like proteins are present in the *Arabidopsis* genome. By applying pressure with

a syringe on the protoplast and by recording the channel activity by patch-clamp, Haswell and colleagues (69) showed that two of these channels respond to mechanical stimuli. In addition to the MscS-like proteins, MCA1, a putative stretch-activated calcium channel, has been identified in *Arabidopsis* (104). Even though the MCA1 protein sequence does not display any significant similarity with any ion channels identified so far, MCA1 is able to complement a yeast mutant impaired in such a channel and that mediates responses to mechanical stimulation (78). *Mca1* mutant roots are unable to penetrate harder growth medium, suggesting a role of MCA1 in transducing a mechanical signal (104).

As shown earlier, there is now accumulating evidence in animal cells showing that the cytoskeleton not only provides a mechanical link between the extracellular matrix and the membrane but also can display a critical role in force sensing (7, 10, 141, 166). Although this seems reasonable in contractile animal cells, it is more surprising to find that, even in walled cells, the cytoskeleton can have an active role in mechanoperception. For instance, in bacteria, the tubulin homolog FtsZ can produce a constricting force (19, 113). Crescentin, an intermediate filament-like protein, has been shown to form a helix when detached from the membrane, as a stretched spring would do after release. Interestingly, it was shown that crescentin is required for the curved shape of *Caulobacter crescentus* and can induce a curved shape when expressed in *Escherichia coli* (19). In budding yeast, atomic force microscopy detected oscillatory motion on the cell wall, which could reflect the action of the cytoskeleton (118). This suggests that the cytoskeleton and associated proteins can exert a force on the cell wall, providing a model in which stress sensing could depend at least partly on the balance between external and internal forces in walled cells, as in contractile cells.

Although the methods of perceiving mechanical stress at the molecular level are diverse, they also seem to display some redundancy. For instance, stretching of detergent-treated cells still leads to the recruitment of focal adhesion proteins onto the cytoskeleton, i.e., when the membrane is dissolved (141). In plants, microtubule reorientation following centrifugation was observed in protoplasts, i.e., when the cell wall was largely digested (167).

SUMMARY POINTS

1. Because morphogenesis is primarily a geometrical output, quantitative imaging protocols have been developed to monitor shape changes in 4-D and at cellular resolution.

2. The cellular mechanisms behind these geometrical changes rely on both biochemical (e.g., the distribution of morphogens) and biophysical (e.g., the elastic modulus of the cell wall) parameters.

3. A plant is a multiscale mechanical structure. Its parts (walls, tissues, organs) can be characterized by their stiffness (elastic modulus) and capacity to yield (extensibility). These parameters are under genetic control.

4. Cells can autonomously control the properties of the walls, possibly resulting in conflicting instructions between neighboring cells or tissues. These conflicts result in mechanical stress, which is predicted to help direct morphogenesis.

5. The combination of micromechanical and live imaging approaches as well as computer simulations support a contributing role of physical stress in controlling growth, cell division patterns, cell fate, and gene expression.

6. Cooperation between biochemical signals, such as auxin, and mechanical forces is likely to increase the robustness of morphogenetic events, such as vein formation or phyllotaxis.

7. Mechanical forces can be perceived via several entry points, such as the cell wall (e.g., wall sensors), the plasma membrane (e.g., stretch-activated channels), and the cytoskeleton.

FUTURE ISSUES

1. There is currently a lack in the measurement of mechanical properties according to cell identity. Attention should be paid to all properties, i.e., yield threshold, extensibility, and elastic modulus.

2. Mechanical stress cannot be observed directly, which calls for the use of multiscale mechanical modeling to infer stress patterns.

3. Mechanical models for growth will undoubtedly become more and more useful in the exploration of hypotheses; however, 3D models are still in their infancy.

4. To test the predictions of the mechanical models down to genetic control, experiments analyzing the impact of forces on known major morphogenetic effectors will have to be performed and the contribution of putative mechanosensors tested.

5. To go beyond a qualitative analysis of the function of the gene regulatory network in morphogenesis, translating the gene input into shape changes, will require the precise quantification of the geometry and mechanical properties of specific gene expression domains in the wild type as well as in corresponding mutants. Development of micromechanical approaches coupled with quantitative imaging techniques will certainly help to achieve this long-term goal.

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Contents