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Title: What shoots can teach about theories of plant form

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Abstract: (110 words)

Plants generate a large variety of shoot forms with regular geometries. These forms emerge primarily from the activity of a stem cell niche at the shoot tip. Recent efforts have established a theoretical framework of form emergence at the shoot tip, that has empowered the use of modelling hand in hand with biological approaches to start disentangling the biochemical and physical mechanisms canalizing form development at the shoot tip. We discuss here how these advances get us closer to identifying the construction principles of plant shoot tips. Considering the current limits of our knowledge, we propose a roadmap for developing a general theory of form development at the shoot tip.

Main text: (4573 words)

Introduction

How organisms shape themselves has been a fascinating and challenging question for centuries. Alan Turing's pioneering work on self-organized pattern formation through diffusion of chemical signals exemplifies the central contribution of mathematical modelling and the impact of a theory on the understanding of developmental biology¹. However, understanding the construction of a form goes beyond patterning (the establishment of the spatial distribution of different cell identities) and must account for the geometry of the living object and the biophysical mechanisms driving geometry changes. The complexity of the problems raised by such integration was already recognized by Alan Turing: “ ... *the description of the [biological system's] state consists of two parts, the mechanical and the chemical. [...] Determining the changes of state [...] is a problem of formidable mathematical complexity. [...] The interdependence of the chemical and mechanical data adds enormously to the difficulty*”¹. In recent years, a massive increase in computational power has finally provided opportunities to tackle this challenge and to test our understanding by creating models that reproduce form development *in silico*, as an emergent property.

Understanding plant forms presents challenges of its own. At the structural level, cell walls as resistant as steel surround plant cells and preclude cell movement. Plants have also unique developmental features that underlie dynamic changes in their post-embryonic form, including continuous apical growth of their axes and reiterative production of new organs and axes throughout their life, and that must be accounted for to understand the emergence of their form. Here we discuss our understanding of form emergence in the tissue that generates the entire aerial architecture of plants, the shoot apical meristem (SAM; Fig. 1). A stem cell niche in the SAM sustains continuous organogenesis. Recent studies of the SAM and aerial organ development have been instrumental for understanding the dynamics of plant morphogenesis at the tissue scale. We review first how computational models have helped establishing the principles of a theoretical framework for morphogenesis at the SAM. Then, we consider recent analyses of how patterns and forms emerge in the shoot apical meristem, focusing on the ones that, combining modelling and *in planta* experiments, have explored and challenged the biophysical mechanisms at play. Finally, we highlight what are in our view the main limitations of the current theoretical view of SAM morphogenesis in order to draw up a possible roadmap for future developments. In complement, we also refer the readers to Box 1 for a short companion guide on how to best use and critically assess computational models such as the ones discussed in this perspective.

A theory of shoot apical meristem morphogenesis

The classical view of morphogenesis in biology assumes a two-phase process initiated by patterning and followed by deformation². These two phases correspond to processes of different bio-physical origins and proposed to act at different temporal scales. In the patterning phase, often considered more rapid than deformation, bio-physical processes create region-defining fields of molecular and physical cues. In the deformation phase, cells read out this patterning information and modify their bio-physical properties accordingly. Patterning thus induces deformation, which in turn, feeds back on patterning by modifying the geometry of the tissue, suggesting that these two processes are deeply interdependent. It also indicates that the classical paradigm of patterning and deformation acting at different time scales needs to be considered with caution. In the SAM, two such phases can readily be identified. They respectively correspond to the dynamic patterning process specifying the initiation sites of primordia of leaves and flowers at the shoot apex, called phyllotaxis, and to the subsequent outgrowth of the organ that generates the typical geometry of the tissue (Fig. 1). In recent years, the elements of a theory integrating the causality between processes controlling form emergence at the SAM have emerged. They are organized according to four main principles (Fig. 2):

Principle 1: “Mobile reference points dynamically organize positional information around a stable central zone to pattern the SAM”

Phyllotaxis is a remarkable example of self-organized patterning. Dynamic positional information is believed to be predominantly responsible for the emergence of the remarkable symmetry of lateral organ arrangement, which is frequently helicoidal (Fig. 1a). No organ can form at the very tip of the plant, which is defined as the meristem central zone. Around the central zone, primordia can spontaneously emerge in an annular region called the peripheral zone. However, it was postulated early on that primordia within the peripheral zone inhibit the initiation of new primordia in their immediate vicinity (For review: ³), preventing organ “crowding”. However, growth progressively pushes young primordia away from the peripheral zone, periodically leaving space for new initiations. Remarkably, abstract models of this dynamic process on continuous templates can recapitulate the frequency and position of these initiations and can generate many of the organ patterns that are observed on plant stems and flower heads ⁴⁻⁶. The central zone acts as a stable reference relative to which the position of the different primordia can be defined. Organs in turn become secondary mobile reference points with respect to the central zone, that refine the positioning of future organs in space and time.

Together they act as a dynamic reference system that continuously reorganizes positional information prescribing the position and initiation time of subsequent organs (Fig. 3).

Principle 2: “Turgor pressure and cell wall remodelling drives growth and deformation”

Once initiated, primordia start to grow. In recent years, efforts to understand the outgrowth mechanism have focused on the origin and role of mechanical forces in driving the changes of form in plant tissues. It was proposed early on that tissue deformation and growth is fuelled by osmosis-driven cell turgor (Fig. 4)⁷. Cells have low water potential (mainly due to high osmotic potential), driving extracellular water uptake to generate high internal turgor pressures. Turgor compresses the cell membrane against the rigid cell walls, which resist both by deforming and by building up internal mechanical stresses. When pressure is moderate, walls deform in a reversible (elastic) manner, but yield irreversibly and are remodelled if the pressure is high enough, leading to growth⁸. In a pioneering work, James Lockhart proposed a simple model for this irreversible mechanism applied to an idealized 1-dimensional (1-D) cell⁹. In this model, turgor pressure is a property emerging from the equilibrium that takes place between the osmotic forces that attract water and deform the cell wall, which in turn resists water ingress¹⁰. The extension of this model to multicellular 3-D structures only emerged recently. First attempts representing 2-D tissues¹¹, then 3D tissues¹², were carried out by modelling wall elements as 1-D springs, and then generalized with elastic surface elements able to better represent wall anisotropy in 2 or 3 dimensions^{13,14}. Using these more general tissue representations, Lockhart’s growth equation could be extended to accurately account for potential directional differences in tissue mechanical properties in 3-D, supporting a pivotal role for turgor pressure and cell walls in regulating growth and deformation of complex tissues such as the SAM (Fig. 4).

Principle 3: “Patterns instruct tissue deformation by regulating cell wall properties and turgor pressure”

To link patterns to form emergence, a conceptual framework building on several pioneering attempts^{15,16} was proposed, marking a key step towards a theory coupling patterning and growth¹⁷. Based on the mathematical fact that any deformation can be expressed using three elementary deformation parameters for each small region (growth rate, growth direction i.e. the principal direction of growth, growth anisotropy i.e. the degree to which growth occurs in any preferential direction), this framework postulated that, at an abstract level, molecular and biophysical cues established during the patterning phase might locally prescribe a “growth plan” in terms of these parameters. In the same way that words can all be constructed from a finite alphabet, elementary deformation instructions could thus be assembled by cells to achieve

tissue growth. Here, the intensity of elementary deformations is directly prescribed by genes, either through scalar values (e.g. molecule concentration) or polarizing factors (e.g. oriented flows or gradients of molecules, cell polarization)¹⁸, and mechanics is used to resolve potential conflicts between neighbouring regions instructed to develop at different paces, and thus to prevent tissues from being torn apart¹⁷. Several studies then attempted to account for local deformations as the result of cellular processes in a more mechanistic way. A first study in virtual cellularised embryos¹⁹ hypothesized that growth results from two independent pathways, one acting on relaxation of cell walls deformed by turgor pressure and a second one that would directly control a hypothetical growth factor locally mediating through yet unknown mechanisms the effect of genes as in ¹⁷. By contrast, other approaches modelled growth uniquely as a result of a cell wall relaxation pathway in tissues with SAM geometry^{13,14}. Here, genes regulate growth by affecting either cell wall rheological properties or cell turgor. The deformation of each wall segment is locally governed by a growth law, corresponding to the Lockhart equation generalized to 3-D (see Principle 2), and whose parameters depend on regional gene expression. This latter view provides a mechanistic framework for the biochemical and genetic control of form emergence in the SAM, positioning the cell wall and turgor pressure as physical actuators controlled by genes (Fig. 4).

Principle 4: “Tissue deformation feeds back on patterns”

However, connections between patterning and growth are not one-way only. In the last decade, it has been discovered that cells in the SAM may sense the mechanical stresses induced by turgor forces in their wall. At the SAM surface, these stresses are a read-out of the local wall geometry that is used by cells to locally modify their biochemical and rheological properties¹². To explore how specific forms can emerge in the SAM from such an intricate interaction and study its properties, further computational models have been developed. They integrate organ geometry, wall rheology, cell turgidity and growth laws with mechanical feedback and have made it possible to simulate forms resulting from different combinations of these factors^{20,21}. These computational models have been and will continue to be instrumental in understanding the properties of complex plant tissues, such as the SAM^{20,21} and in further exploring the general principle that morphogenesis is finely regulated by a feedback loop between patterning and deformation (Fig. 2, 4). As pointed out previously, this last principle implies that patterning and deformation cannot be easily temporally separated.

Identifying mechanisms to test the theory

Recent studies combining modelling and *in planta* experiments have been essential in

identifying molecular mechanisms linking signals, genes and form emergence, and at the same time for testing the current theoretical framework for morphogenesis at the SAM.

The stem cell niche acts as a stable reference point

In *Principle 1* of the theory, the tip of the SAM acts as a stable reference, essential for defining the coordinates of future growth axes and thus for form emergence. Functionally, this reference is the central domain of the SAM containing the stem cell niche (Fig. 1b). The stem cells are located in the SAM in the external cell layers at the very tip of the stem axis. An organizing centre that maintains the niche is located below. These two domains control each other through a homeostatic feedback loop (Fig. 5a; for review: ²²). Organizing centre cells produce the WUSCHEL (WUS) transcription factor, which moves and activates the *CLAVATA3 (CLV3)* gene. CLV3 peptides then diffuse to inhibit *WUS*. Beyond its apparent simplicity, this regulatory loop raises a difficult question: how are *CLV3* and *WUS* expression domains maintained in a growing tissue one on top of the other? Models of the WUS/CLV3 pattern on a static template provide a basic understanding²³⁻²⁹. The WUS-CLV3 loop recalls the activator-inhibitor couple in a Turing system¹, but with the activator and inhibitor produced by different cells. The fact that *CLV3* is always expressed above *WUS* within the shoot tip can be explained by including epidermal-derived signals (Fig. 5a). Conversion of cytokinin precursors into bio-active cytokinins (for review: ²²) and/or a mobile miRNA³⁰ are candidate mechanisms. Direct interaction of WUS with the HAIRY MERISTEM (HAM) transcription factors could inhibit the capacity of WUS to induce *CLV3* in the organizing centre, allowing WUS to activate *CLV3* only when it moves in the outermost layers where HAMs are absent. HAM activity could thus also participate in the separation of *WUS* and *CLV3* expression domains and to the confinement of *CLV3* to the outer domain^{28,29}. HAM expression patterns could again depend on epidermal signals. The most recent models have included this idea and, using realistic 3-D growing templates, suggest that epidermal signals could indeed ensure that the WUS-CLV3 pattern remains stable despite shoot growth (Fig. 5b)^{28,29}.

To act as a stable reference, the stem cell niche must not respond to organogenetic signals. As in other plant tissues^{31,32}, the plant hormone auxin is an instructive signal driving patterning in the SAM. Auxin is polarly transported from cell to cell. While auxin distribution in the SAM sets the locations of lateral organ initiation (see below), auxin is also present at high concentration in the central stem cells niche (Fig. 5c)³³⁻³⁵. However stem cells are largely insensitive to auxin^{34,36}, likely due to a down regulation of auxin signalling transcriptional effectors, the Auxin Response Factors (ARF), in the centre of the SAM^{33,37}. Ordinary differential equation modelling of the ARF-dependent signalling network confirms that

differential expression of ARFs could lower sensitivity of the stem cells to auxin³³ and ARF mis-expression in the stem cells shows that this spatial sensitivity pattern is essential for phyllotaxis³⁷. Stem cell auxin sensitivity regulation depends on WUS, which, together with other transcription factors, gates auxin signalling through gene repression^{38,39}. The maintenance of a SAM reference point for patterning is thus intricately linked to a spatio-temporal regulation of responses to developmental signals. Collectively, these studies identify multiple biochemical signals and gene expression regulation systems that establish the SAM central reference point hypothesized in *Principle 1* of the theory.

Auxin gradients patterns the shoot apical meristem

Spatio-temporal regulation of auxin distribution appears to be the main biological mechanism both explaining lateral inhibition by organs and triggering growth of new organs outside the SAM central domain^{33,35,40-43}. Cooperatively with other auxin carriers^{42,44}, the efflux carrier PIN-FORMED1 (PIN1) is a polar protein essential to the control of polar auxin transport. PIN1-mediated supra-cellular fluxes in the SAM are essential for organ formation and for phyllotaxis⁴². Live-imaging of PIN1 polarities and auxin distribution in several plant species, and extensive modelling work, suggest that self-organization of PIN1 polarities allows not only the local accumulation of auxin, specifying new organ sites, but also the emergence of inhibitory fields, by depleting auxin in surrounding cells^{33-35,45-52}. Thus, positional information hypothesized in *Principle 1* of the theory is provided by auxin (Fig. 5c). However, the mechanisms organizing PIN1 polarities are still elusive. Computational models based on two different mechanisms, auxin flux sensing or auxin cellular concentration sensing, can both reproduce realistic PIN1 polarity patterns^{49,53}, making it difficult to pinpoint how PIN1 polarities are coordinated within the SAM. Also, adaxial-abaxial patterning of lateral organs might feedback on auxin polarities⁵⁴, raising the interesting possibility that identity specification during the development of lateral organs dynamically changes their influence on PIN1 polarities. A recent study that quantitatively mapped PIN1 polarities and auxin distribution in 4-D now provides opportunities to re-evaluate and extend existing models³⁵. This study further revealed that cells in the peripheral zone integrate auxin information over time to trigger organ initiation (Fig. 5c). Temporal integration of auxin information was predicted by a recent inhibitory field model with stochastic induction of organ initiation⁵ developed to explain the occurrence of perturbed phyllotactic patterns⁵⁵, but still needs to be integrated into cellular models with precise SAM geometry. Furthermore, existing mechanistic models do not explicitly take into account the high auxin concentrations present at the centre of the SAM. This appears to be an important missing element since a recent study combining live imaging with a

simplified compartment model to represent functional subdomains of the meristem, showed that high auxin levels in the centre of the SAM are essential for maintenance of stem cell niche size, and that lateral organs are required to maintain these high auxin levels⁵⁶. The recent identification of young flower primordia as putative sites of auxin biosynthesis and the demonstration that PIN1-mediated fluxes could converge globally towards the central zone auxin maximum³⁵ corroborate this finding. Together, these studies highlight the role of auxin in defining the central zone as a stable reference and in establishing lateral organs as secondary mobile reference points as postulated in *Principle 1*. These two properties are thus interdependent and emerge from auxin-dependent regulations that are still under investigation (Fig. 5c).

Cell wall mechanical properties are modified during organ growth

How does pattern become form? *Principles 2* and *3* of the theory predict a central role for the cell wall. Cell wall anisotropy is directly linked to cellulose microfiber orientation⁵⁷. In their absence, cells tend to grow isotropically, like soap bubbles⁵⁸. The extracellular deposition of cellulose microfibrils by Cellulose Synthase (CESA) transmembrane proteins is directed by dynamic cortical microtubules (for review: ⁵⁹). Cellulose microfibril patterns thus resemble those of microtubules in SAM cells⁶⁰. Auxin has been shown to induce a loss of microtubule anisotropy prior to lateral organ initiation⁶¹. Numerical simulations using cell-based models predict that changing both cell wall anisotropy and stiffness is required for organ emergence^{61,62}. Indeed, loss of cell wall anisotropy is tightly coupled to the induction of cell wall-loosening enzymes, through an yet unknown mechanism⁶². This cell wall anisotropy/loosening module creates a direct molecular link between cell identity specification and the regulation of cell wall mechanical properties (Fig. 6). The loosening could be mediated by enzymes that modify cell wall polysaccharides, notably those that compose the cellulose-embedding visco-elastic matrix. Accordingly, micromanipulations and mutants in genes encoding several such enzymes perturb phyllotaxis⁶³⁻⁶⁶. Physical measurements using atomic force microscopy, or manipulation of osmotic pressure, show differences in stiffness and elasticity between the SAM centre and periphery^{65,67,68}, indicating differences in cell wall mechanical properties matching SAM functional zonation. Although genetic approaches have not identified all the enzymes responsible (possibly due to functional redundancy), these data nonetheless support the hypothesis that changing cell wall mechanical properties (stiffness, elasticity, anisotropy) is a primary function of genes driving form emergence (*Principle 3*).

Mechanical feedbacks monitor form changes

The existence of mechanical feedbacks from *Principle 4* of the theory was first highlighted by a combination of imaging, genetics and modelling that demonstrated that cortical microtubules align with the direction of maximal tensile stresses in the SAM¹². Modelling confirms that axial growth is consistent with a feedback law in which tissues produced by the SAM rigidify preferentially in the maximal tensile stress direction, and is incompatible with a law where tissues rigidify in the direction of maximum elongation^{12,20}. How mechanical stresses are sensed in the SAM remains unclear, although the microtubules could themselves act as sensors⁶⁹. Microtubule anisotropy results in cell wall and thus growth anisotropy. This is in turn reinforced in response to mechanical stresses resulting from changes in form. Interfering with this feedback affects meristem geometry and particularly crease formation at the boundary between SAM and new organs⁷⁰. Furthermore a combination of live-imaging and modelling suggests that PIN1 polarity distribution can be affected by mechanical stresses⁷¹. Analysis of *cesa* mutants reveals that impairing cellulose synthesis alters both microtubule organization and the auxin-dependent expression of PIN1⁶⁰. Inducing changes in cell wall mechanical properties through enzymatic modification of the pectin matrix affects PIN1 polarity distribution⁷². Osmotic treatments and modelling also demonstrated that mechanical strain positively regulates the amount of PIN1 present in membranes, notably in lateral organs at the SAM⁷³. Together these data strongly support an essential role for mechanical feedbacks in allowing constant form monitoring and pinpoint auxin as a key mediator linking mechanics of the tissue to cell/tissue physiology. Nonetheless, other mechanisms still need to be considered in the future, e.g. involving other hormones. In addition, form can also feedback on the expression of meristematic genes, likely via auxin-independent mechanisms^{26,74}.

The limits of the theory

Missing elements in the theory

Combining biological and modelling approaches, has permitted a significant step change in our understanding of SAM form emergence over the last decade. Our conceptual understanding of form emergence nonetheless remains fragmented. Biochemical and mechanical mechanisms have largely been considered independently, as have patterning and form acquisition. A key challenge is now to quantitatively analyse coupling of biochemical and mechanical regulations to build a more integrated theory of form emergence. Auxin is a common regulator acting at multiple scales in these mechanisms and provides a possible entry point to a unifying biophysical vision of form emergence at the SAM. Analysis of coupling between biochemistry and mechanics at the SAM will also be key to understand form robustness, i.e. reproducibility

between SAMs despite the existence of noise. There are now clear examples of how noise is buffered or contributes to form emergence in the SAM. Noise in the rhythmicity of organ patterning^{5,75,76} can perturb form. At the same time, mechanical feedback mediated by microtubules amplifies growth differences, generating variability in cell size and maintaining a robust form at the SAM⁷⁰. A role for mechanical resistance to growth of the epidermis has also been proposed in generating robust tissue shapes⁷⁷. A plausible hypothesis is that form emergence is not only influenced by, but also uses both structured and random changes in geometry and mechanical stress distribution as cues for the dynamic generation of form. Integrating noise into the theoretical framework of SAM form emergence will allow this idea to be tested.

Further development of a theory of morphogenesis dynamics at the SAM requires a deeper understanding of the cellular mechanisms that drive cell growth and division in a tissue context. Progresses in quantitative imaging have permitted to test the influence of cell geometry on division plane orientation in various tissues⁷⁸. In the SAM, mechanical signals have been proposed to contribute to division plane orientation (notably in the organ boundary region; Fig 1b) in addition to geometry, thus providing an understanding of how the tissue context can act on this process^{79,80}. Two studies have also explored the control of cell division and expansion in the SAM, demonstrating that cells do not simply divide when they reach a critical size (a simple hypothesis often used in models), but that there is instead a flexibility in the cell size reached at division^{81,82}. However, we know very little about either the role of cell division orientation or indeed even of how subdivision of tissues into cells can influence the biophysical properties of tissues and thus form emergence. These questions have just started to be addressed⁸³ (building on earlier work: ⁸⁴) and require further experimental and theoretical analysis. Variation in SAM form between genetic variants has been genetically associated with genes regulating either cell division or expansion, highlighting the importance of considering the cellular scale in a theory of form emergence at the SAM⁸⁵. Also, the recent demonstration that cell contact topology in the SAM depends on microtubule-mediated mechanical feedback, and could determine the transport network topology of auxin⁸⁶, provides a first possible mechanism for coupling geometric/chemical/mechanical feedbacks acting on cells to tissue-wide patterning and form emergence.

Understanding the control of the mechanical properties of the SAM and their dynamics remains challenging. Atomic force microscopy and a physical model of pressurized cells have recently been used to measure turgor pressure in the SAM epidermis, opening the possibility of direct evaluation of the contribution of turgor pressure to form emergence⁸⁷. Turgor pressure is

dependent on both cell wall elasticity and water fluxes between cells. Both this study and a theoretical analysis suggest that water fluxes provide a coupling mechanism between cells that can regulate growth and form emergence^{87,88}. Several studies have suggested the existence of developmentally-regulated symplastic sub-domains in the SAM^{89,90}, where plasmodesmata connectivity could allow for movement of solutes and water between cells. Symplastic connectivity could have a significant impact on turgor pressure regulation and turgor and mechanical forces in general have been suggested to influence plasmodesmata aperture (⁹¹⁻⁹³; see also ⁹⁴ for further discussion). Understanding turgor pressure regulation during form emergence in the SAM will thus likely require considering a combination of inter-dependent apoplastic and symplastic regulations. In relation to this, most analyses have concentrated on the epidermal layer. However, mechanical properties of the internal layers could be important for organogenesis⁶⁵ and the establishment of the vasculature in the internal tissue might also affect the hydraulic properties of the SAM, in addition to a potential involvement in regulating auxin distribution^{48,50,51}.

Finally, very recent data on leaf pavement cells suggest that the self-organization properties of pectin polymers in the cell wall could contribute to cellular growth, due to their orientation and to a swelling independent of turgor pressure ⁹⁵. This idea is quite intriguing and will need to be consolidated, both from the experimental and theoretical point of view (see ⁹⁶ for an in depth discussion). Nevertheless, it points to the importance of expanding our understanding of the biochemistry of the cell wall (which is still a challenge), in addition to the exploration of the turgor pressure regulation. Future advances will thus require model development allowing further quantitative analyses, the inclusion of water hydraulics, ions and osmoregulation, cell connectivity and a more detailed 3D tissue representation taking into account the cell wall structure in a theoretical framework.

A need for new modelling and biological tools

As illustrated by the work discussed in this perspective, the elaboration of a general theory of plant morphogenesis increasingly relies on the development of intensive computer simulations⁹⁷. They make it possible to predict what forms may emerge from putative laws driving the interaction between plant constituents or cells. To proceed further, new simulation platforms will need to be developed to program and simulate the development of complex forms in a flexible environment, and would be the counterpart for multi-cellular tissues of the successful approaches used for modelling the development of plant architectures with L-systems at a more macroscopic scale⁹⁸⁻¹⁰¹. These platforms should provide ready-to-use high-level tools to describe forms or infer them from microscope data, to write and run development

simulation programs on them, to visualize the simulated forms and their properties at various scales and to assess the simulation results against real data.

Complementary to this and as highlighted in Box 1, it is indeed essential to be able to quantify biological parameters in order to properly build and evaluate the validity of model predictions. Pushing the frontiers of our understanding of form development is then dependent on the generation of new biological tools, imaging and image analysis methods. We have for example discussed how biosensors have allowed quantifying the spatio-temporal distribution of auxin³⁵. Numerous biosensors allowing to follow the distribution of a variety of biochemical components in cells and tissues are already available and more are undoubtedly to come. This constitutes a unique toolbox that still needs to be used to its full potential. On the contrary, biological tools allowing direct measurement of mechanical forces at the cellular and tissular scales are largely missing in plants. Fluorescent sensors for mechanical parameters, including turgor pressure, have been developed in animal systems¹⁰² and similar approaches could be considered in plants to extend the available toolset to measure mechanical properties of plant living tissues.

Finally, most of the work considered here used *Arabidopsis thaliana* for wet experiments. Generating a general theory of plant shoot forms will require extending the number of species that can be used experimentally and modelled, which is an important challenge for the entire plant developmental biology community.

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CONTRIBUTIONS

T.V, F.B and C.G designed the figures and wrote the manuscript. F.B. constructed the figures.

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FIGURE LEGENDS

Figure 1: Morphology and functional organization of the shoot apical meristem. The SAM of the main inflorescence of *Arabidopsis thaliana* is shown. **(a)** Top view (z-projection) image of a SAM with fluorescently-stained cell walls obtained using confocal laser scanning microscopy. The round structures bulging at the periphery in a regular arrangement are the growing flower primordia (P), numbered from youngest to oldest (P0-10). **(b)** Reconstruction of a longitudinal section going through P0 and P4 (dashed white line in (a)) showing the clonal organization of cells in layers and the typical dome-geometry of the SAM. The stem cells (yellow), primordia (blue) and primordium-meristem boundaries (red) are shown.

Figure 2: Principles of dynamical form emergence at the shoot apical meristem. The SAM changes over time with the development of new flowers on its flank while maintaining a central dome where the stem cells are located. The green longitudinal sections on both sides illustrate the changes. In the centre, boxes identify the four principles guiding the dynamic self-organized emergence of this complex shape (see text).

Figure 3: Inhibitory field-based model of phyllotactic patterning at the shoot apex. Phyllotaxis can be modelled with organ-centered inhibitory fields (blue discs, radius= d) moving away at the surface of a growing meristem with a dome geometry (green); top views: a-c; lateral views: b-d. Lateral organs are numbered from the youngest one. The specification of P0 at the boundary of the stem-cell domain (yellow, radius R) as a result of growth is illustrated.

Figure 4: Theoretical multiscale view of form emergence at the shoot apex. A schematic *Arabidopsis* SAM bearing two primordia is depicted during the initiation of a new primordium (P0). Zooms of the P0 area and of a cell and its cell walls are shown. Form emerges from several biochemical/physiological (here morphogen gradients and gene expression) and physical (here mechanical stresses; arrows indicate intensity and direction of stress anisotropy) signals influencing growth. These signals feedback on each other and collectively create a dynamical biophysical positional information field instructing cell deformation, controlling changes in cell wall rheology and possibly turgor pressure. A simplified representation of mechanical stress in cell walls is shown using springs.

Figure 5: Molecular mechanisms of patterning at the shoot apical meristem. (a-b) The shoot stem cell niche is stably positioned at the shoot tip despite constant apical growth. **(a)** WUS (purple) and CLV3 (yellow) interact through a regulatory feedback loop. Two cells in the

WUS domain are represented. Shoot growth displaces the expression domains and replaces constituent cells. P: primordium. **(b)** Models suggest that epidermal-derived signals anchor the WUS-CLV3 pattern in the growing shoot tip with a contribution of HAM (see main text). X1 is a long-range WUS-activating factor (such as cytokinin), X2 a short-range WUS inhibiting factor (such as a miRNA) and X3 a short-range HAM inhibiting factor. **(c)** Mechanisms allowing auxin to control patterning at the SAM.

Figure 6: Molecular processes acting in tissue deformation during primordium initiation.

A schematic meristem with a dome geometry is depicted without organs to show how primordium form emergence is regulated and changes mechanical stress distribution (arrows indicate relative intensity and direction of stress anisotropy). Left: at the site of P0 emergence, a region of auxin maximum (red) appears next to a region of auxin minimum (blue). In each region, zooms of a cell and cell wall are shown. The intensity and direction of mechanical stress is the same in the two regions. At the auxin minimum, microtubules align to the principal stress direction, guiding cellulose synthesis by CESA and precluding growth in the main stress direction. At the auxin maximum, auxin uncouples microtubule orientation from stress direction, so that new microfibrils are deposited in all directions. Wall-loosening enzymes, induced by auxin and other factors, reduce the stiffness of the cell wall matrix. Cell growth is then faster and isotropic in the high auxin region (Right). The emergence of the primordium changes mechanical stress distribution, feeding back on primordium development.

Box 1 (650 words)

A short guide to computational models: strengths and limits

Like all models, computational models are simplified representations of the reality with which one can reason or operate. They are a convenient tool to think the real, without being overwhelmed by its intrinsic complexity.

Computational models have both a structure and parameters. The structure reflects the main hypotheses used to construct the model: biological elements described as components or compartments of the model, type of interaction laws between these elements, dependence/independence of processes, time and space scales, etc. The parameters make it possible to adapt the structure to the modelled object: kinetic constants of molecular interactions, thresholds, material stiffness and/or viscosity, coupling constants between biophysical mechanisms, transport coefficients, etc.

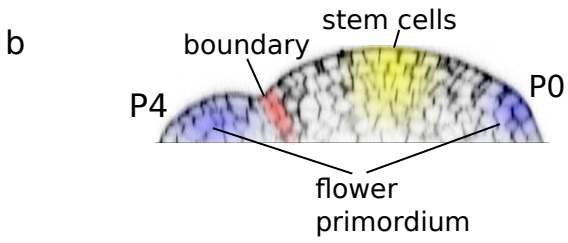
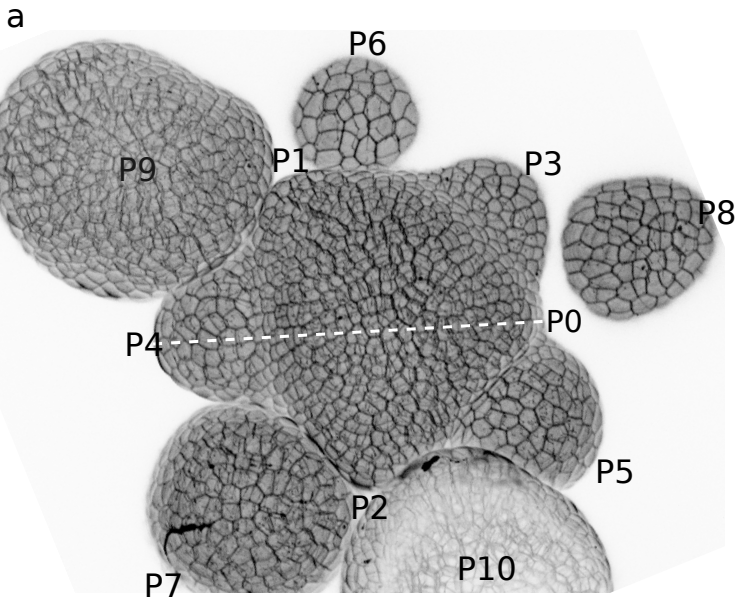
The most sensible question when evaluating a model is not whether it is right but whether it is useful. This can be for different reasons. First, the design of a model's structure can be instructive as it can pinpoint the lack of knowledge in parts of the modelled system, and suggest new experiments. Second, simulations can show that no parameter of the chosen structure may account for the observations. This also is informative as it leads to reconsider the model's structure and hypotheses (for instance replace a 2D model of organ geometry with a 3D one). Alternatively, the model can confirm that there exists a plausible range of parameters that shows that the hypothesized model structure does capture the observations well enough. The various models of auxin transport in the SAM (see main text) for instance, successfully confirmed that phyllotaxis patterns can emerge from putative rules controlling PIN1 allocation to membranes in a growing tissue as a function of auxin fluxes and local concentrations.

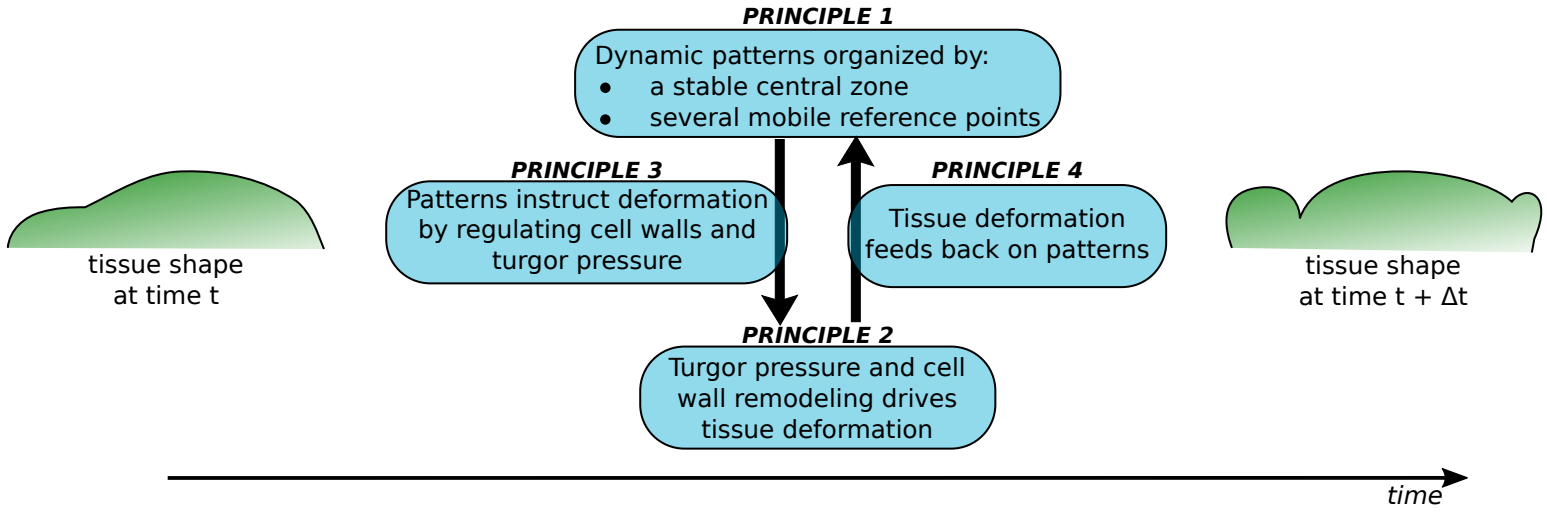
Another essential consideration to judge the usefulness of a computational model is whether it is a faithful representation of the observed phenomenon. Arguments may rely on its parsimony (i.e. its structure captures the studied phenomenon in a concise but efficient manner), on the fact that its structure reflects that of underlying physical/chemical mechanisms and that the used parameters have been measured or estimated in a realistic range, on whether its emerging mathematical or numerical properties reflect correctly those of the observed phenomenon or on its predictive power, i.e. its ability to predict observations that have not been used to construct the model and that possibly have not yet been observed.

One must however be cautious: reproducing real observations *in silico* is a necessary, but not sufficient, condition to be a useful model or to say something about the real. It may be that the phenomenon could be reproduced with a model that does not reflect the actual underlying physical or chemical processes. For example, it is still possible that yet another set of rules controlling PIN1 polarization, not yet identified, provides convincing simulations and better reflect what actually happens in a growing SAM. It may also be that the number of parameters is so large, that one does not learn much on the observed system as it is always possible to simulate sufficiently well a phenomenon by using a sufficiently high number of parameters. To avoid this, different techniques are used e.g. define parameters so that they can be measured experimentally or use known observations to find parameter values that can explain the observations (called model inversion and correspond to a form of indirect, model-assisted measurement, e.g. used in ⁴⁵). Finally, the model may also only partially reproduce known facts:


as pointed out in the main text for example, none of the models of auxin transport in the SAM currently reproduces the observed accumulation of auxin in the CZ.

Like microscopes are necessary tools to see the invisible, computational models are powerful tools to disentangle the complexity of a system. They contribute to exploring the real, pinpoint new questions, suggest experiments and help reasoning and testing hypotheses but the interpretation of their results must always be critically assessed.





stable central zone



inhibitory fields produced by organs

