Computational Morphodynamics: A Modeling Framework to Understand Plant Growth

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Abstract

Computational morphodynamics utilizes computer modeling to understand the development of living organisms over space and time. Results from biological experiments are used to construct accurate and predictive models of growth. These models are then used to make novel predictions that provide further insight into the processes involved, which can be tested experimentally to either confirm or rule out the validity of the computational models. This review highlights two fundamental challenges: (*a*) to understand the feedback between mechanics of growth and chemical or molecular signaling, and (*b*) to design models that span and integrate single cell behavior with tissue development. We review different approaches to model plant growth and discuss a variety of model types that can be implemented to demonstrate how the interplay between computational modeling and experimentation can be used to explore the morphodynamics of plant development.

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INTRODUCTION

Systems biology has emerged as a field that attempts to integrate large-scale datasets obtained from genomics, gene expression, proteomics, metabolomics, and imaging studies into a global framework to explain cellular and organismal function (75). These systems approaches integrate the biological sciences with the quantitative approaches of applied mathematics, physics, and engineering to explicitly model biological processes computationally. A computational model provides an explicit formulation of a hypothesis that allows one to simulate, predict, and generate visualizations of biological processes. The use of models to explain biological processes is not new to biology. In the past, however, such models have been largely conceptual, schematic-based descriptions that, although they may adequately describe all relevant interactions, lack the ability to generate precise, quantitative predictions, and, furthermore, cannot predict the emergent properties of complex systems (8). A computational model is more adaptable and allows one to integrate the two-way interaction between geometry and cellular/molecular function over space and time and make precise, verifiable predictions. Furthermore, such models can be rigorously fit to a variety of biological data. Thus computational morphodynamics has emerged to explain complex temporal and spatial interactions of growth and signaling through the use of computational modeling integrated with biological imaging.

Realistically modeling plant growth is challenging because it occurs on several scales, which range from the cellular to the tissue level, and ultimately to a consideration of the whole plant, in which the emergence of organs dictates overall form. Two key challenges to modeling plant growth are (*a*) to integrate chemical or molecular models with mechanical models to create a self-organized growing template and (*b*) to create models that describe the development of complete tissues or organs in terms of single-cell dynamics. A computational morphodynamics study begins when researchers extract cell geometry and topology information to create a mechanical cellular template from a biological image (**Figure 1**). Data from genetic, biochemical, cell and molecular biology, and imaging experiments are used to infer the biochemical network that controls developmental signaling processes. The model is constructed such that the biochemical network lives inside each cell and directs interactions between those cells. A feedback loop ensues between the mechanical properties of single

cells and the biochemical network within each cell. Through this loop, signaling can influence cell growth, and cell growth can feed back to influence signaling processes. Finally, the model can make dynamic predictions that are used to generate new hypotheses, which can be tested experimentally.

The use of mathematical equations to explicitly describe biological processes in model form allows for a greater exploration of intuitive ideas and the generation of computer models that are easier to visualize. Computational morphodynamics seeks to uncover general principles by exploring mathematical models based upon experimental observations. To achieve this, we believe that models should have the following characteristics: (*a*) models should be biologically based and explicit—variables described in the model should have counterparts observed in the experimental data that the model will be calibrated against; (*b*) models should be parameterized with biological data when available; and (*c*) models should be built to result in key predictions that are experimentally testable.

Models are organized based on one of two approaches: bottom up or top down. The bottom up method combines key components such as interacting genes, proteins, and metabolites to build mathematical models of reasonably small networks through loss- or gain-of-function perturbation experiments (49). In contrast, a top down approach integrates available data at the genome level to construct large networks (61). Nonetheless, both approaches use some common modeling methodologies. In this review, we begin with examples of the models that were developed to explain plant growth. Then, we describe the methodologies used to construct these models. Next, we take an in-depth look at the field of computational morphodynamics and highlight the fundamental contribution of live imaging to the formulation of realistic computation models. We also propose two key challenges that must be addressed by the field of computational morphodynamics and discuss progress being made toward meeting these challenges. Finally, we look forward and discuss some future directions for the role of computational morphodynamics in developmental biology.

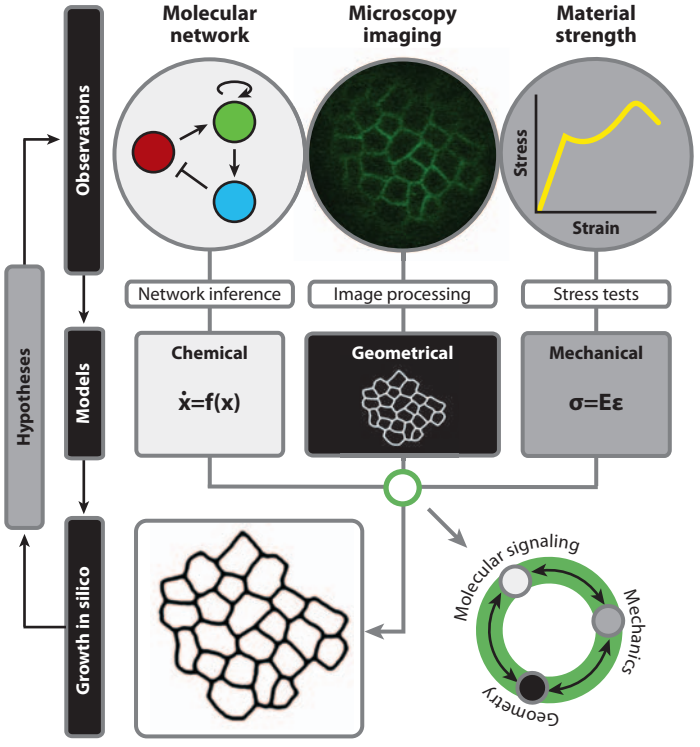

Figure 1

Schematic of a proposed computational morphodynamics experiment. An hypothesis is tested by the experimental flow diagrammed (*at left*). Data are collected from experiments to determine molecular, geometrical, and mechanical parameters of a computational model. The three components are interdependent. Finally, the model is used to make new predictions to further modify the initial hypothesis, which can be retested experimentally.

MODELING EXAMPLES OF PLANT DEVELOPMENT

Plant growth has been examined on all levels, from the single cell to the whole plant, and corresponding models have been created. These models employ different approaches at various levels of complexity. Here we illustrate some of these through examples.

A Kinematic Approach to Dynamics

A kinematic approach first measures the growth of the organ or plant through imaging and

then uses that information to build a computer model that reproduces the growth without addressing the underlying mechanisms that cause growth. Unlike dynamic models, these models abstract such details as activities of cells and the molecular framework underlying the growth decisions (93, 127).

There are two major classes of kinematic models. The first class attempts to replicate the growth of the plant as a whole. Two such models describe the growth of *Arabidopsis* and rice plants (84, 129). Extensive measurements of growth were used to fit parameters to models based upon L systems, which are a set of formal grammars that describe the reiterative nature of plant growth (95, 96). The plant models are divided into modules, e.g., leaf, meristem, internode, and flower, and are produced iteratively according to the observed development of the plant. These models are able to reproduce *Arabidopsis* and rice plants that bear a striking resemblance to plants found in nature.

Whole plant models are useful for predicting how environmental factors such as nutrient availability or crowding will affect the overall growth of the plant (55). When applied to crop plants, kinematic models have agronomic value in predicting optimal growth conditions. For example, Hiller et al. used models and measurements of maize leaves to show that the reduction in leaf growth in the upper leaves of densely grown plants is caused by a reduction in the duration of the linear expansion phase, not a change in the growth rate (55).

The second class of kinematic models takes measurements of growth in different regions of the organ and predicts the resulting shape of the organ. These models are often based on the inference of growth from the analysis of clonal sectors induced at different times during development. For example, Rolland-Lagan et al. used sectors in snapdragon (*Antirrhinum majus*) induced at early stages of development to infer the growth rates in the petal lobe (103, 104). The growth model is parameterized by the growth rate, anisotropy (different growth rate in one direction versus another), and direction with respect to an external grid. All of these can vary in space and time. Based upon the measurements, Rolland-Lagan et al. constructed a spring model for the growth of the petal lobe to show that the crucial parameter in generating the asymmetry of the lobe is the growth direction. Their model led them to predict the existence of a long-range signal that acts to orient growth vertically throughout the petal.

Kinematic models can lay the groundwork for future models in which the growth and proliferation of individual cells, regulated by molecular signaling networks, recreate the growth of the plant. As a starting point, molecular decision-making networks can control the developmental decisions that were hardwired into these descriptive models initially. For example, Prusinkiewicz et al. took a step in this direction by adding a gene regulatory network that includes the key regulators LEAFY (LFY) and TERMINAL FLOWER (TFL) to an L-systems model to control the decision of whether a meristem becomes a shoot or a flower (94). They show that by varying LFY and TFL activity, they can reproduce the inflorescence architecture of wild-type, mutant, and overexpression plants. Similarly, Coen et al. expanded their model of snapdragon petal growth (see above) to associate differences in growth patterns with regional identities that correlate with the expression patterns of genes such as *CYCLOIDEA* (*CYC*) and *DICHOTOMA* (*DICH*) (15). This 2D finite element model correctly reproduces the petal shape of both wild type and petals in which CYC is overexpressed. However, neither of these models incorporates cellular mechanics, which is one of the challenges for the future.

Biomechanics of Single Cells

In contrast to kinematic models, single-cell models focus exclusively on individual cells as the basis for growth. These models of plant growth assume that wall loosening leads to a drop in turgor pressure. This causes the movement of water into the cell to maintain the turgor pressure and leads to irreversible cell

expansion (74, 113). Anisotropic growth, as discussed by Baskin et al., has some dependence on cellulose microfibril alignment (4). An early model proposed by Veytsman et al. described extension of cell walls due to interactions between wall polymers (126). The deposition of cellulose fibrils in cell walls is oriented by cortical microtubules; therefore, the dynamics of microtubules are an essential consideration in models of cell growth (18, 90, 128).

Dumais et al. studied tip expansion in *Medicago truncatula* root hairs using measurements of wall expansion rates in conjunction with a mechanical model of the elastic properties of a pressurized thin shell (28). The expansion patterns obtained from the model led to the conclusion that the observed anisotropic tip expansion was caused by anisotropy of the mechanical properties of the cell wall. Although these types of models can help us understand single-cell morphodynamics, we need multicellular models to comprehend macroscopic plant patterns.

Mechanics of Tissues

Plant tissues are composed of connected cells, with the constraint that cells cannot slide past each other (65). The growth of individual cells occurs through the mechanisms discussed above. In tissue models, cell walls that are under stress due to the turgor pressure are in quasi-static equilibrium, which over longer timescales get lengthened because of the stress. Recent work suggests this stress can lead to the local rearrangements of the underlying microtubules, thereby modifying the direction of cellulose deposition, which can feed back to influence the stress on cell walls leading to anisotropic growth (50).

The appearance of primordia in the peripheral zone of the shoot apical meristem has been hypothesized to arise as a result of buckling (26, 117). Dumais et al. used a pressurized shell model based on observations from gaping patterns caused by cuts in the sunflower SAM to predict stress patterns and concluded that primordia could be initiated in the generative zone by buckling caused by compressive stress (30).

Recent models have also attributed venation patterning to mechanics of the underlying tissue (10, 68). Alternative models describe vein patterning as the result of the distribution of the phytohormone auxin in tissues (25, 118). The role of the mechanical properties of plant tissue has also been used to explain the rapid dynamics of the Venus fly trap and the twining of plants (35, 45, 56).

SAM: shoot apical meristem

Examples of Chemical or Molecular Signaling and Gene Regulatory Network Models

Numerous signaling components and gene regulatory networks govern plant growth and development, but few have been modeled computationally. The following section describes those chemical and molecular signaling pathways and gene regulatory networks that have been modeled, which highlight the usefulness of predictions obtained from those models.

One of the most extensively modeled systems in plant development has focused on the transport and signaling of the phytohormone auxin. Auxin is an essential regulator of plant development that controls cell identity, cell division, and cell expansion, which are primarily dependent on its distribution in plant tissues. Indole-3-acetic acid (IAA) is a weak acid and a predominantly naturally occurring auxin that is subject to ion trapping upon entering cells. The PIN-FORMED (PIN) gene family of auxin efflux facilitators mediates the directional transport of the auxin anion throughout tissues by providing channels, asymmetrically distributed in the plasma membrane of plant cells, through which auxin can exit the cells (102). Modeling auxin transport at the tissue level has provided important insight into the role of this hormone in several biological processes.

Patterning of the plant vasculature is dependent on the flow of auxin. Sachs conducted experiments that led him to propose that plant veins form along auxin flow paths because of positive feedback between auxin flux and auxin

PIN1: pinformed 1

transport capacity (106). Sachs's model suggests that cells with an increased capacity for transport act as sinks. This was subsequently modeled by Mitchison, who proposed that if the concentration within a transport route decreased with increasing flux, then auxin would move into these cells and form the transport route (80). However, experimental data show that the auxin concentration in developing vascular tissues is higher than in surrounding tissues, which is required for vascular development (106). In response to this criticism, Mitchison proposed that cellular auxin concentrations only need to be low during the initial stages of vascular canalization, when cells are acting as sinks. As development proceeds, the presence of an auxin anion channel that is localized to one end of the cell, while being depleted from the other end, could maintain the flux of auxin throughout a cell even if the cellular concentration was high. Two recent modeling studies support this hypothesis and suggest that auxin could accumulate under the conditions proposed by Mitchison (34, 36). Imaging studies on the localization of PIN1 (the PIN isoform found in developing vein cells) in developing veins have confirmed several aspects of PIN polarity predicted by the flux model. The orientation of PIN1 during the initiation of tertiary veins resembles the localization of PIN1 in simulations based on the flux model in which a new vein forms from the sink towards the source (110). Experimental results show that PIN1 accumulates initially in a cell adjacent to the secondary vein and is subsequently expressed in the next adjacent cell farther down from the source. In these cells, the localization of PIN1 is directed towards the pre-existing secondary vein as supported by the flux model. A separate modeling study found that simulations run with a simplified flux model incorporating auxin sources could generate realistic patterns of vein formation (41, 78, 105).

In the shoot apical meristem, auxin plays an important role in the initiation of new organs. The first indication of the establishment of a new leaf or floral primordium is the formation of a peak of auxin, which preceeds any morphological or gene expression change. The high concentration of auxin in this peak in turn drives both the gene expression changes and the outgrowth of the primordium (88, 101, 102). Several mathematical models that are based on experimental data have been proposed to predict auxin flux and PIN1 polarity patterns during organ initiation. Live-imaging studies on developing inflorescence meristems demonstrated that PIN1 is largely confined to the epidermal layer of cells in the shoot apical meristem and is initially polarized toward the area of tissue with the highest auxin concentration, where the incipient primordium is located. However, as that primordium becomes established, PIN polarity reverses in cells at the edge of the primordium and reorients toward the next primordium (54). Several new computational models have been developed that describe PIN1 polarity patterning during primordial induction; these models suggest that a positive feedback occurs between cellular auxin concentrations and the direction of auxin transport as new primordia are specified (22, 59, 119). All these models suggest cell-cell signaling as a way to coordinate PIN1 polarity toward the cell with the highest auxin concentration, thereby specifying new floral primordia. One model predicts the presence of an auxin maximum at the apex of the meristem (22). This was shown experimentally by the strongest immunolocalization signal of auxin in the central zone of the shoot apex and developing primordia and the presence of the auxin influx mediator, AUX1, in the epidermal layer of the shoot apical meristem (22, 102). These data suggest a situation in which auxin is transported apically into the meristem such that a maximum forms in the central zone of the epidermis initially and auxin is transported outward and basally through the action of the PIN1 efflux mediator in the specification of new organ primordia subsequently.

During development, cell identity can be specified by two nonexclusive mechanisms: cell lineage specification by genetic determination and positional information perceived by

molecular or other signals that are nonuniformly distributed throughout the organism. In plants, the interpretation of cues that signal positional information is often the predominant mechanism for acquiring cell identity (111). The leaf and root epidermis in *Arabidopsis* bears specialized hairs that form in distinct patterns. In the leaf, hairs (trichomes) are spaced in dotted patterns with no clustering, whereas the root epidermis is divided into alternating cell files of trichoblasts (hair cells) and atrichoblasts (nonhair cells). The patterning of hair cells in the *Arabidopsis* epidermis is controlled by a genetic network that involves the regulation of *GLABRA1/GLABRA3/ENHANCER OF GLABRA3* genes in the leaf and *WEREWOLF/GLABRA3/ENHANCER OF GLABRA3* in the root. These activator complexes are inhibited by the function of two genes, *TRIPTYCHON* (*TRY*) and *CAPRICE* (*CPC*) (69, 112). Two recent papers have reported computational models of this genetic pathway, which provide useful predictions about its structure that were experimentally verified subsequently.

Benítez et al. developed an activation-inhibitor system, a type of Turing reaction-diffusion system (see section below) to model the *GLABARA1* (*GL1I*) and *WEREWOLF* (*WER*) genetic networks (7, 91). They found that despite the presence of *GL1* in the leaf and *WER* in the root, the activator complex that formed with *GLABRA3 (GL3)/ENHANCER OF GLABRA3 (EGL3)* in both genetic networks functioned as predicted by their model simulations.

This model produced a pattern of spaced, not clustered, trichomes similar to that observed in the leaf epidermis. This activation-inhibition system was based on a reaction-diffusion system that specifies local self-activation and lateral inhibition. In the root, epidermal cells that overlie two cortical cell layers develop into trichoblasts, whereas those that are above one cortical layer develop into atrichoblasts (37). Genetic evidence points to the function of a membrane receptor protein kinase, *SCRAMBLED* (*SCM*), in sensing a signal derived from the cortical layer in epidermal cell specification (66). Benítez et al. updated their reaction-diffusion model to include a signal from the cortical layer that would specify epidermal cell identity. Model simulations only produced the observed pattern of alternating hair and nonhair cell files when the signal from the cortical layer was modeled to induce the *WER* activator complex. This suggests that the signal from the cortical layer has an important function in the induction of the epidermal cell fate through the induction of the WER activator complex.

Recently Savage et al. challenged the activation-inhibition system as the underlying mechanism for patterning in the root epidermis (108). Benítez et al. assumed that WER is self-activated, which had not been determined experimentally. Savage et al. proposed two different models that centered on the mode of regulation of *WER* in an epidermal interaction network based on a stochastic Boolean formalism. The first model assumes that local *WER* self-activation with CPC represses *WER* indirectly (local WER self-activation model), whereas the second model does not include *WER* self-activation but assumes uniform *WER* transcription that is repressed by CPC and SCM activity (mutual support model). The mutual support model of the *cpc* mutant closely matches the experimental observation of increased *WER* expression in *cpc* trichoblast cells (69).

The authors then determined experimentally that *WER* expression is the same in a wild-type or *wer* background, which rules out local WER self-activation. The mutual support model also correctly predicts the wild-type *WER* expression pattern in the *gl3/egl3* double mutant background. These data provide direct experimental support for the mutual support model, which rules out *WER* self-activation as a mechanism for epidermal patterning in the root and highlights how computational model predictions can guide the formulation of new experiments to elucidate the function of biological pathways.

Combining Signaling and Mechanics

ODE: ordinary differential equation

The coupling of molecular signaling and mechanics is a challenge that has provided insight into several different aspects of plant growth and development. Two systems that have been considered from this perspective are phyllotaxis and pollen tube growth.

Shipman et al. presented a phyllotaxis model that combines biochemistry with growth mechanics (86). Instead of placing auxin in discrete compartments to approximate the properties of cells, the authors treated auxin as though it had a continuously variable, position-dependent concentration throughout the meristem. Mechanically the meristem was modeled as a compressed sheet that buckles to minimize energy, similar to a previous model by Shipman and Newell (117). The results of this hybrid model suggest that the primary determinant of phyllotactic and meristem patterns can be the auxin concentration, compressive stress, or the cooperative interaction between biochemistry and biomechanics.

Pollen tube development is another area that has received ample attention by modelers. Denet et al. modeled molecular signaling by the diffusion of morphogens in the pollen tube; Bolduc et al. described the mechanics of pollen tube growth using finite elements; and Dumais et al. used a viscoplastic system (11, 23, 29). These models considered biochemistry and mechanics separately, but Kroeger et al. combined both aspects (64). In this model, the pollen tube is represented as two fluids separated by a curved interface that simulates the cytoplasmic membrane. The growth of this interface is related to the relative internal pressure and the elastic properties of the wall with a hydrodynamic equation. A unique element to this model is the inclusion of calcium diffusion, which is coupled to membrane dynamics by a diffusion equation that changes in response to the elasticity of the membrane and simulates a stretch-activated ion gate. This close coupling of mechanics and molecular signaling generates the oscillatory pollen tube growth observed in nature. The model is plausible and needs to be tested by further experiments.

So far, these computational amalgamations of biochemistry and mechanics have provided useful insight into the morphodynamical processes of plant development. We hope that models continue this trend as the field progresses.

MODELING METOHDOLOGIES

In this section, we describe the methodologies used to create the models discussed above. First we look at the molecular modeling techniques that describe the interaction between the regulators of growth and plant development. Second we describe methods for producing the growing cellular templates.

Molecular Modeling

Molecular models exist in a wide variety of mathematical representations, which include differential equation models, stochastic models, Boolean models, Petri-nets, cellular automata, and event-based models (20, 79).

The choice of the model depends to some extent on the type of data available. The description of biochemical networks in terms of ordinary differential equations (ODEs) is a common approach in systems biology (122). Examples of ODE models in *Arabidopsis* include models of circadian clock dynamics and ethylene signaling (24, 73, 134). Differential equations describe the evolution in the concentration levels of participating species such as mRNAs, proteins, and small molecules. Models are constructed based upon known or assumed interactions and require experimental parameters such as the stoichiometry and reaction rates. At the most fundamental level, reactions can be described in terms of mass action kinetics, and approximated with Michaelis-Menten, Hill-function based, or more complicated mathematical expressions, which include power functions (S-Systems) and sigmoidal functions (neural networks) by assuming rapid reactions and high concentrations of the participating species (81, 109, 114). In Hill-like equations, exponents are indicators of

the cooperativity of the system. In some models, gene regulatory networks assume that the binding and unbinding of transcription factors to DNA is rapid, compared to other processes; this allows a thermodynamic approach that has been used to map out gene regulatory function, which is essential for understanding how inputs from transcription factors at each gene are integrated to build a network (9, 63). Analysis of the network dynamics reveals several features of the system, which include multiple steady states for protein levels, or oscillations in protein levels, and the time it takes for the system to settle down after perturbation. These features can be addressed by the application of nonlinear dynamic techniques such as bifurcation theory and by traditional engineering techniques such as perturbation analysis (120). Biological networks are replete with interacting negative and positive feedback loops, and these methods can be used to extract useful properties of dynamics that inform us about the functionality of the network (125). We can search networks for motifs (a subnetwork which has particular dynamical properties) that can often impact the functionality of the whole network (2, 76).

The differential equation approach can be extended to model spatial dynamics by using reaction-diffusion equations (85). We assume that individual cells are tiny compared to the dimensions over which the reacting species diffuse. These equations are then solved in continuous space with appropriate boundary conditions. As originally hypothesized by Turing, pattern formation can occur by an activator-inhibitor interaction (39, 124). The activator induces itself and a second molecular species. The second molecule feeds back to inhibit the original activator. Both the inhibitor and activator are produced in the same location. However, if the inhibitor diffuses away from the activator rapidly, then a peak of activator can form. This was the approach taken in the trichome and root hair models proposed by Benítez et al. (see above) (7). Such an approach has also been used to describe the pattern of *WUSCHEL* expression in the shoot apical meristem where the authors extracted data from confocal imaging to parameterize their model (58). The reaction-diffusion approach is a good approximation if the transport of network components occurs through passive diffusion. However, we need a cellular framework for plants to explain the polar transport of auxin out of a cell by PIN proteins that are localized to specific domains of the plasma membrane (107). We can compare cellular descriptions with experimental data easily if the simulations are performed on extracted cellular templates from imaging, which is another compelling advantage of the use of models that explicitly include cells (59).

Stochastic fluctuations in molecule numbers caused by the inherent randomness of transcription and translation, degradation events (intrinsic noise), and environmental signals (extrinsic noise) have an effect on the dynamics of the underlying genetic network (60, 97, 121). There are now numerous examples of networks that either utilize noise or are adversely affected by it. Although such an approach is not used broadly in the plant biology community yet, it is still important because a theoretical treatment may uncover novel functionality of a network in terms of its ability to filter out or amplify noise.

Boolean models are used for large networks in which we often have incomplete information (3). In this case, the simplest assumption to make about the activity of a gene is that it is either on or off. At each node (gene), all inputs into that node are integrated into a logical rule such as OR, AND, or more complicated regulation. For a network of interacting genes that is initialized at the start, a synchronous update gives the state at the next time step. Asynchronous updates simulate differences in temporal biological processes (1). The dynamical behavior of such networks can be stable, critical, or chaotic. Using this framework, we can study the stability of the network in terms of the gene regulatory rules that occur at every gene. For large networks in which little information is available about components, such an approach is computationally feasible as compared to an ODE model. Recent models of *Arabidopsis* gene regulatory and signaling networks using this formalism address the ABC

model of flower development, abscisic acid regulation of stomata closure, and the patterning of the root epidermis (32, 71, 108).

FEM: finite element method

Mechanical Modeling

We need a proper mechanical description of cells to provide an accurate description of growth. In the weak spring models, each cell has walls (shared by neighboring cells) that are composed of weak springs joined at the vertices (82, 116). The springs are considered visco-elastic elements that are under tension due to the turgor pressures on either side of the cell wall and maintain equilibrium by balancing the resulting forces on very fast timescales. Growth is described quasi-statically by gradually increasing the spring resting lengths on a slower timescale. Cell division occurs by introducing a new cell wall to partition a cell into its two daughters when it reaches a threshold volume. Spring models have been used to describe cell growth and proliferation during the establishment of phyllotaxis, auxin-induced growth, growth of cells in the SAM, and leaf venation caused by mechanical forces (16, 17, 31, 59).

Alternatively, the growth of cells can be described in terms of stress and strain relationships using the theory of elasticity. Specifically, Dumais et al. used thin shells with the mechanical property of anisotropic visco-plasticity to describe tip growth in plants cells, which included root hairs and pollen tubes (29).

A third approach is to use the finite-element method (FEM), which is a commonly used technique in engineering (5, 87). FEM involves solving the partial differential equations of elastic media by first partitioning the domain of interest into smaller elements, which each have a simple polyhedral shape. Within each element, an approximation of the stress-strain relationship allows one to solve for the displacement and impose the continuity of solutions across element boundaries. In regions where stress and strain gradients are high, more elements are required to capture the rapidly changing behavior of the system accurately. Recently, Hamant et al. used the FEM to model stress-strain in the L1 layer of the shoot apical meristem and correlated this to the behavior of the cortical microtubule arrays of these cells (50).

The cellular Potts model is another method to study the growth and proliferation of cells using a stochastic framework (6, 40, 47). A given configuration of cells is defined by an energy function in terms of the shape and size of its cells. The energy is reduced in small, stochastic steps as a cell grows to its target area. Grieneisen et al. used such a formalism to describe the growth and division of cells in the root as a function of varying levels of auxin by making the cell cycle time a function of the auxin concentration (48).

There are several other multi-scale computational frameworks, each of which can serve as a substrate for modeling regulatory networks within each cell in addition to intercellular communication. These frameworks deal with individual reactions at the smallest scale and organ growth at the largest scale. Recent examples of computational frameworks include L-Studio simulation, OpenAlea software platform for virtual plants, CellModeller software, and Dynamical Grammars (82, 92, 96). The Dynamical Grammars framework can represent discrete entities such as cells with mechanical attributes that allow for growth and division (82). Within each cell, gene regulatory networks are described by either ODEs or by stochastic processes. Furthermore, the diffusion of molecules allows for intercellular communication. The grammars are rules that represent transitions of various types, such as cell growth, biochemical reactions within cells, the movement of molecules from a cell to a neighboring cell, and cell division.

IMAGING AS A FOUNDATION FOR COMPUTATIONAL MORPHODYNAMICS

Computational morphodynamics seeks to uncover the dynamic feedback between the mechanics and molecular processes that occur in development. Live imaging is the first step to follow the growth and development of cells as

they form tissues and organs. From the resulting images, we can infer molecular mechanisms that regulate growth and form the basis of the multicellular mechanical template in which the molecular networks reside. Next, we form spatio-temporal mathematical models to test hypotheses through dynamic simulations of growth. Finally, model-predictions are validated by further imaging experiments. In this section we discuss imaging and image processing techniques that are the basis for building computational models. In the subsequent section on computational morphodynamics, we provide examples that demonstrate the integration of modeling and imaging.

Live Imaging—Capturing Growth As It Occurs

Live imaging is simply the repeated imaging of a living sample over time that captures the dynamic process of growth and development (100). One technique for tissue-scale live imaging is scanning electron micrographs of dental resin impressions made from living shoot apical meristems (27, 67). These images show beautifully the growth of epidermal cells and changes in the curvature of the meristem as primordia emerge. However, these images are limited to showing the shapes of cells on the surface. To visualize both subcellular structures and underlying cells, live imaging has been conducted with confocal laser scanning microscopy (12, 53, 98). In confocal imaging, transgenic plants that express fluorescently tagged proteins can be imaged. Ubiquitous nuclear or plasma membrane markers and stains are useful to determine cell division and growth patterns (12, 52, 98). Likewise, the dynamics of gene expression patterns and protein localization can be correlated in space and time with live imaging (54). We expect additional microscope technologies such as multi-photon microscopy, which allows greater imaging depth, and digital scanned light sheet fluorescence microscopy, which allows rapid imaging with low phototoxicity, to bring added resolution and power to plant live imaging experiments in the future (33, 62).

However, the live imaging technique presents several challenges. The first challenge is to access the tissue of interest. For exposed tissues, such as roots, this is not a problem; but for tissues that are covered, such as the shoot apical meristem, accessibility can be a major issue. In the case of the inflorescence meristem, overlying floral buds are delicately dissected away prior to imaging (100). Alternatively, primordia can be removed from the meristem by treating plants with the polar auxin transport inhibitor NPA. When these plants are removed from NPA, they recover, initiate primordia, and can be imaged (46).

The second challenge is fitting a living plant under the microscope. In some cases, such as the imaging of a moss, this is straightforward because the plant can still grow mounted in water on a slide (52). In other cases, the part of the plant of interest is attached to a slide and mounted with a cover slip while the rest of the plant grows normally (**Figure 2*a***). Alternatively, the whole plant can be grown in a plastic box, submerged in water, and imaged with a water dipping lens (100).

The third challenge is keeping the plant alive and healthy during imaging. Restricting the amount of laser light to which plants are exposed is crucial because photobleaching affects the marker being imaged, and phototoxicity often kills cells. Various tissues and particular fluorescent markers have differential sensitivity and this is generally determined experimentally. Staining the tissue with a vital dye such as propidium iodide can mark the occurrence of dead cells. Plants being imaged should be inspected after imaging to ensure they are still growing at normal rates (12, 100). In addition, most plants must be removed from the water, dried, and placed back in the growth room in a normal vertical orientation between imaging sessions to allow normal growth. Some plants die in the process, and their data are eliminated from consideration. When clones of cells and division patterns that are observed in live imaging match clones that are generated in sectoring studies, this indicates that live imaging is not altering the normal growth of the plant (52, 98).

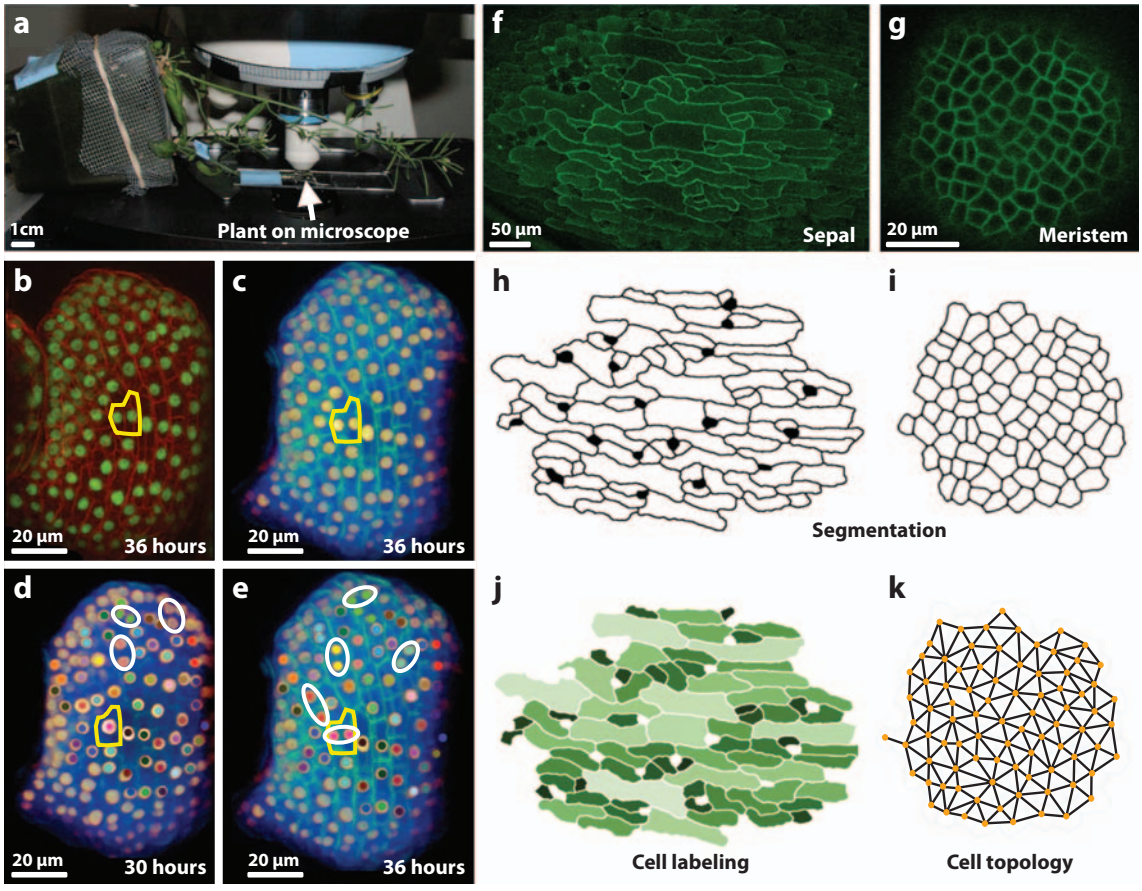

Figure 2

Live imaging and image processing. (*a*) To live-image sepals, the inflorescence (arrow) was taped to a slide and mounted under a cover slip in water. The whole plant was tipped on its side in a covered pot such that the inflorescence was positioned to observe the side of the sepal. (*b-e*) Live imaging was used to examine the cell division pattern in *loss of giant cells from organs* (*lgo*) mutant sepals. Epidermal nuclei (ATML1::H2B-mYFP) and cell walls (propidium iodide) were fluorescently labeled. The sepal was imaged every 6 hours for 78 hours. Cells from the pink lineage were outlined in yellow in all images. (*b*) A projection view of the *lgo-1* mutant sepal at the 36-hour time point was made using Zeiss LSM microscope software. Nuclei are shown in green and cell walls in red. Note that surrounding flower buds are also visible. (*c*) Volume rendering of the same 36-hour image using Amira software. Nuclei are shown in gold and cell walls in green. Note that adjacent flowers have been cropped and the rotation of the sepal has been changed slightly through registration with other sepals in the time series. (*d-e*) Cell lineages were manually tracked from the 30-hour (*d*) to the 36-hour (*e*) time point. Cells in a lineage were marked with the same colored dot. Daughter cells immediately after division were circled in white. Note that the pink cell at 30 hours divides horizontally to make two daughters at 36 hours. (*f-k*) Cells were segmented (*b-i*) from plasma membranes of a projection of sepal epidermal cells (*f*) and a confocal section of a shoot meristem (*g*). The segmented cells can be analyzed to determine their area (*j*) (light colors for larger cells) and their connections (*k*). Scale bar in *a*: 1 cm, *b-e*: 20 µm, *f*: 50 µm, and *g*: 20 µm (A. H. K. Roeder, M. Heisler, A. Cunha, and E. M. Meyerowitz, unpublished data).

The selection of the time interval for imaging depends on the speed required to capture the process as it is changing. For example, imaging every six hours is sufficient to catch cell divisions in the shoot apical meristem and track cell lineages but longer timeframes are not (98). PIN protein localization tends to be more dynamic, and two-hour intervals are more appropriate (54). In contrast, events that occur during the regeneration of new meristems from callus

can often be tracked over daily intervals (44). The selection of the interval also depends on the requirements of the system to keep the laser light low because imaging too often damages the plants.

Some biologists may wonder whether live imaging is necessary given its labor-intensive nature and difficulty. For decades, biologists have inferred processes that happen in time from dead and fixed samples taken from multiple plants at different times. These images can be beautiful and are ideal for automated image processing (123). However, although this approach has certainly been successful in answering static questions, it cannot show any dynamic processes. One example of the value of live imaging comes from studies whose goal is to elucidate the processes that determine meristem size. Although it had long been known that *clavata3* (*clv3*) mutants have enlarged meristems, it was not known whether the enlargement was caused by proliferation or incorrect cell fate specification (14). Through live imaging of plants while transiently knocking down CLV3 activity, Reddy et al. show that cells are first incorrectly respecified and only subsequently is proliferation altered (99). This experiment shows the advantages of live imaging and the value of transient interventions in determining the function of genes. The results have been incorporated into a recent model that describes the homeostasis of meristem size (38).

Image Processing

The primary role of image processing and analysis is to reveal the shape and size of objects of interest in microscope images and to identify the spatio-temporal relationships among them. Image processing produces quantitative data that ultimately serve as empirical evidence in the design, validation, and optimization of growth models. With accurate measurements, one can build realistic computational representations that mimic the geometry of cells and their network of connections to form the basis for mechanical molecular models (**Figure 1**). Furthermore, image processing provides a detailed geometric description of the cells, tissues, and organs that are necessary for the resizing and reshaping of cells as they divide and grow, which can form the basis for growth in the model. The procedures in image processing include (1) visualization of the data, (2) segmentation, (3) registration, (4) tracking, and (5) creating realistic geometry for a mechanical model.

Visualization of 4D Data

Visualizing a 4D dataset produced by live imaging generally requires specialized software. Although useful, projections generated by confocal microscope software packages or ImageJ are insufficient (**Figure 2*b***). Image analysis packages with volume rendering capabilities such as Amira (Visage, GmbH), Paraview, and Imaris (Bitplane, AG) allow the creation of 3D visualizations that can be rotated freely and cropped (**Figure 2*c***) (51, 115). Contrast enhancement, reduction of noise, deblurring, and similar procedures can enhance images in preparation for segmentation (extraction of features) and provide a richer visualization experience.

Segmentation—Extraction of Features from Images

The computation of shape and size follows from a deterministic image-processing pipeline in which segmentation plays a major role. Image segmentation is the process of partitioning an image into distinct regions, each representing a single homogeneous object (42). It is a fundamental step in the quantification of morphology because it generates digital masks from which one can calculate volume, area, length, and shape properties for each segmented object (**Figure 2*f*–*k***). Segmentation is also called on when computing the connectivity (topology) between objects because we need to detect them before finding their neighbors. Topology gives us the adjacency of cells for any given cell in a tissue, which is essential information when investigating cell-to-cell communication.

Extracting meaningful information from an image is not a straightforward task. Often

one needs to craft a specific image-processing pipeline to analyze a particular set of images. Practical and theoretical limitations to image formation, acquisition, and algorithm development prevent universal image processing solutions. For example, images of plant tissues that are generated with a confocal microscope might have a diverse range of characteristics that would make the design of a general and robust (error-free) processing algorithm challenging, if not impossible. This is in accordance with the *No Free Lunch* ideas that state that a general-purpose, universal optimization strategy is impossible and that specialization is necessary (130). Nonhomogeneous illumination patterns, the presence of different levels of noise throughout, missing signals in crucial areas, bad alignment of time lapse frames, and mismatches during image mosaicing are a few problematic issues that are difficult to detect and repair automatically. Too many factors lead to unpredictable image quality and aberrations that directly impact the design of robust image processing algorithms.

A small amount of human intervention is often required to correct automatic segmentation results because there is rarely enough knowledge built into algorithms to tackle all possible variability. This semiautomated approach should not be confused with a manually generated solution (e.g., completely segmenting a cell by hand), because it is a minimal intervention that provides a computer with essential missing information.

Segmentation has been used in roots and shoots to extract cells. Marcuzzo et al. propose an automatic 2D cell segmentation method applied to the *Arabidopsis* root (77). They apply the watershed segmentation algorithm followed by a classification step to discard segmented regions that are not cells. Their SVM (support vector machine)-based classifier is trained with a cell contour descriptor. Results show a reduced false-positive rate of segmented cells compared to the pure watershed segmentation results. de Reuille et al. propose a cell segmentation and reconstruction protocol to extract cell geometry and topology in the L1 surface layer of the *Arabidopsis* SAM (21). They reconstruct the outer surface of the L1 layer such that only cell walls visible from outside the meristem are present in the projection image. Then, junction vertices where cell walls meet are manually marked and grouped for each cell. Finally, an automatic procedure determines the polygonal cell topology and maps the 2D sheet of cells to the 3D surface of the meristem.

Registration: Alignment of Images and Computation of Growth

In a live imaging session, often the experimenter physically moves the sample, or the growth of the tissue changes the orientation, so that images from different time points do not directly align. For example, after moving the stage significantly between images, alignment of the quiescent center provides the static reference point for growing roots (12). Although every effort should be made to preserve the alignment between imaging sessions, subsequent computational registration or alignment of the images is generally required. In its essence, a registration method computes the geometrical transformation that is necessary to completely align (superimpose) two consecutive images of the same growing object. This transformation can be done without allowing the image to be stretched to produce a time series to visualize growth (**Figure 2*d*,*e***) (52). Alternatively, images can be warped to align object boundaries. This warping shows how much stretching is required to deform the object so that its shape is the same in both images, which provides the biologist a measure of growth rate. Image registration thus offers a measure of absolute growth and preferential growth direction (83).

Cell-Lineage Tracking

Segmentation is often the primary step in the computation of cell lineage because we need to locate and enumerate the cells present in each frame of a time-lapse image precisely before associating mother and daughter cells. Human tracking of cell lineages is an

extremely laborious and time-consuming process. (**Figure 2*d*,*e***). Therefore, several programs have been designed to track the lineage of cells automatically (43, 57, 70, 72). This may be another good application of the semiautomated approach in which a computer offers a plausible solution, which is verified by the user and corrected by a biologist if necessary.

Translating Imaging to Realistic Geometry for Models

The reconstruction of cell geometry and topology from images enables us to build finite element meshes needed for plant tissue simulation. The transition from cells represented as a collection of unlabeled pixels in images to cells represented as finite element meshes is transforming the way we analyze images for predictive simulation (132, 133, 135). Now, with varying levels of difficulty, we can reconstruct cells from images and create complicated simulations with their geometry. Efforts to bring this methodology to the computational studies of plant development are discussed below.

TWO CHALLENGES OF COMPUTATIONAL MORPHODYNAMICS

The advent of live imaging (covered in the section above) has dramatically impacted our approach to computational modeling and allows us to address the two main challenges of computational morphodynamics. The first challenge is to understand how local molecular signaling interacts with mechanical growth processes to change behaviors of individual cells. The second challenge is to determine how the sum of the behaviors of individual cells culminates in the overall size, shape, and form of each organ in the plant. If we integrate these models into a single understanding successfully, we can predict how changing the subcellular signaling within the cell will alter the morphology of the organ and test this prediction with experiments. Tremendous progress has been made in the past few years to address these challenges. The next few studies highlight the role of experimentation in the formulation and subsequent validation of the model.

Two recent papers have demonstrated a role for mechanical signaling in the morphogenesis of the shoot apical meristem in *Arabidopsis* during development (17, 50). Hamant et al. utilized physical and mathematical approaches to demonstrate that SAM morphogenesis is dependent on the orientation of the microtubule cytoskeleton, which is regulated by mechanical stress on cell walls in the epidermal cell layer. Live imaging of cell morphology by laser confocal microscopy that followed depolymerization of the microtubule cytoskeleton with oryzalin revealed that cellular differentiation and the patterning of new primordia were not dramatically affected. However, the loss of the microtubule cytoskeleton blocks cytokinesis, which results in the formation of giant polyploid cells with a geometry that resembles two-dimensional foams that are isotropic in nature. The notable effect of these changes is the absence of the crease that forms between the meristem and primordia and suggests that although microtubules are not required for developmental patterning, such as phyllotaxis, they are required for morphogenetic effects such as tissue folding. Further analysis of microtubule orientations revealed that at the meristem apex, microtubule orientations are highly dynamic, whereas those at the base and periphery of the meristem align in circumferential radial arrays primarily. At the boundary between the flower primordia and the meristem, microtubules are aligned in supracellular orthoradial arrays along the boundary domain.

Previous studies provided a link between maximal stress directions and the orientation of cortical microtubules (131). The anisotropy of stress across a plant cell wall is caused by the direction of the rigid cellulose microfibrils that are laid down during cell wall synthesis; and because this often parallels the direction of cortical microtubule orientation, cellulose microfibril synthesis may be guided by cortical microtubule tracks (13, 19, 89). To determine the relationship between stress on cell

walls and the orientation of microtubules, a mechanical model was developed to calculate the direction of principal stresses in different domains of the meristem. The meristem was modeled as a shell inflated by internal pressure that depends on three assumptions: (*a*) the outer wall in the epidermal cell layer supports turgor pressure, (*b*) limited growth, and (*c*) uniform pressure from the inside. The principal stresses calculated from this model were found to be parallel to the cortical microtubule orientations observed in live images from meristem tissue. The authors went a step further and designed a spring model stretched over a 2D curved surface that approximated a meristem-like shape. This model contained visco-elastic wall mechanics, stress feedback, growth and proliferation, and microfibrils, which indicated the direction of cellular mechanical anisotropy. The model reproduced the observed microtubule orientation in living tissue on all templates tested, which include the orthoradial cortical microtubule orientation at the boundary between floral primordia and meristem. The model also reproduced the loss of the crease between the boundary of primordia and the meristem and ballooning of cells in the pin-shaped template when the anisotropy was removed for the parameters of the model that simulates oryzalin treatment.

Two approaches were taken as a final test of whether the orientation of microtubules along the principal direction of maximal stress can alter the orientation of cell wall synthesis and thereby regulate morphogenesis. The first approach utilized laser ablation of a single cell or multiple cells to locally eliminate turgor pressure that would induce changes to the stress and strain on cell walls in the central zone of the L1 layer of the meristem, in which cortical microtubule orientations and growth patterns suggest cells walls are mechanically isotropic. Theoretical predictions, obtained by modeling the L1 layer using the finite-element method and ablation simulated by a loss of turgor pressure and reduction of the elastic properties of ablated cell walls, indicated that a rearrangement in principal stress directions occurs after cell ablation such that principal stress directions shift gradually from radial to circumferential around the wound site. Confirmation of these model predictions was obtained by laser ablation studies of cells in the central zone of the L1 layer followed by live imaging, where microtubules reorient circumferentially around the ablation site. As a second and final approach, the authors applied force directly to the meristem tissue and observed the orientation of microtubules by live imaging. Upon meristem squeezing, cells at the apex of the meristem either stabilized or aligned their cortical microtubules toward the axis that is parallel to the maximal stress direction. These results indicate that stress control of microtubule-controlled anisotropy of cell wall synthesis, which in turn reinforces cells against principal stress directions, is required for certain morphological events such as tissue folding and maintenance of a cylindrical stem uncoupled from control of differentiation and growth.

In a separate study, Corson et al. observed isotropic cell growth after microtubule depolymerization in a growing shoot apical meristem, similar to the results of Hamant et al. (17). Under these isotropic growth conditions, they also observed that the angle distribution between cell walls, where they meet at a vertex, was similar to the angles observed between vertices of soap films, which indicates that cells were at mechanical equilibrium with each other. In addition, the authors observed that cells with <6 sides have convex cell walls, whereas those cells with >6 sides have concave cell walls in oryzalin-treated meristems. Next, they performed simulations of a simplified two-dimensional model of growing cells, each of which is maintained in elastic equilibrium over short timescales against turgor pressure by cell walls modeled as viscoelastic rods. When they completed simulations of the mathematical model that assumed uniform turgor pressure in all cells, the cell shapes did not match the experimental data obtained by live confocal imaging of oryzalin-treated meristems. Instead, simulations of a model assuming turgor is correlated to the size of the cell correctly predict the geometry of the observed

cell shapes. Some of the larger cells with a higher number of cell walls have a concave shape, whereas the smaller cells are often convex, which fits the live confocal imaging data from oryzalin-treated meristems. This suggests a link between cell size and turgor pressure, which the authors hypothesize is a general feature of plant tissues that maintains a level of homogeneity of cell size to generate a template for other patterning mechanisms to operate.

A recent study implicates a molecular to mechanical connection in roots. Grieneisen et al. used a Potts framework to describe the mechanics of a growing root, in which auxin transport caused by PIN is simulated (48). Confocal imaging of PIN protein patterning was used to build a mathematical model of auxin transport on a geometrical template of cells. The regulated auxin transport patterns the growing root to have a maximum of auxin located in the stem cell region. The authors showed that the auxin maximum occurs because of the change of direction of auxin flow, when basal flow through the vasculature towards the root tip turns around to flow back up through the epidermal cells apically. The auxin maximum was robust to the transport parameters of the model, auxin production and decay, and tissue ablation. A link between the mechanics and signaling is hypothesized, such that cells in the neighborhood of the auxin maximum proliferate rapidly; whereas farther away from this center, where the auxin gradient decreases, cells grow longer. The faster growing cells, which sense a shallower gradient of auxin, move away from the rapidly proliferating center, thereby giving rise to a sharp delineation of meristematic and elongation zones, agreeing well with observations.

SUMMARY

As we discuss in this review, there are two key challenges to producing predictive computational morphodynamic models of plant growth. The first challenge is to integrate molecular models with the mechanics of growth to create a self-organized growing tissue. To meet this challenge, first we must perform biological experiments to discover the key molecular components that determine the growth phenomenon being studied, i.e., relevant genes, hormones, and the interaction network. Following the detailed dynamics of cells using live imaging allows us to extract a cellular template, which can form the basis of a computational model. Then we need to couple the mechanical properties of simulated cells with the molecular network. The second challenge is to scale from single cells to organ scale growth. To meet this challenge, we propose using live imaging data to follow single cells and their contributions to whole tissues. Although innovative steps toward both challenges have already been taken, more advances are needed to reach the goal of a fully computable plant.

FUTURE ISSUES

1. Image analysis should be 4D. A step in this direction will involve advances in the live imaging technique, either in the optical configuration or in the way a sample is imaged, to obtain better resolution in the deeper parts of the tissue. When we can truly track all of the cells, we can begin to match their growth with their spatio-temporal gene expression patterns to gain insight into the molecular mechanical feedback loop.
2. Models also need to be 4D. These models should be built with finite elements to model the whole tissue of growing and dividing cells. For this purpose, finite element software needs to be more adaptive to describe growth and cell division.

3. One technical challenge is that finite element model (FEM) mesh elements become unstable when the mesh grows, and elements increase their size by several orders of magnitude. The resulting remeshing required is computationally expensive. To calibrate the FEM wall properties, we need to extend our analysis of cell wall growth. In addition, FEM software needs to interact with molecular modeling software such that the molecular network can modify properties of cell walls and the structure of the cellular framework. For example, each cell should include a molecular cell cycle model that can regulate spatially dependent cell division rates.
4. We need to develop a combined simulation environment in which we can iteratively add many different types of models to describe the progression of several developmental processes. Because many groups contribute to the increasing pool of available mathematical models, such a modeling environment would serve as a platform to integrate and exchange models.
5. Successful computational morphodynamics studies require the assembly of multidisciplinary teams including biologists, image processing experts, and modelers. We believe computational morphodynamics will provide a platform for future collaborations between biology and the computational sciences to solve the beautiful mysteries of plant growth.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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LITERATURE CITED

1. Albert I, Thakar J, Li S, Zhang R, Albert R. 2008. Boolean network simulations for life scientists. *Source Code Biol. Med.* 3:16
2. Alon U. 2006. *An Introduction to Systems Biology-Design Principles of Biological Circuits*. Boca Raton, FL: Chapman & Hall/CRC Press, 320 pp.
3. Alvarez-Buylla ER, Benitez M, Davila EB, Chaos A, Espinosa-Soto C, Padilla-Longoria P. 2007. Gene regulatory network models for plant development. *Curr. Opin. Plant. Biol.* 10:83–91
4. Baskin TI. 2005. Anisotropic expansion of the plant cell wall. *Annu. Rev. Cell Dev. Biol.* 21:203–22
5. Becker EB, Carey GF, Oden JT. 1981. *Finite Elements*. Englewood Cliffs, NJ: Prentice-Hall. v. 1, 258 pp.
6. Beltman JB, Maree AF, de Boer RJ. 2007. Spatial modelling of brief and long interactions between T cells and dendritic cells. *Immunol. Cell Biol.* 85:306–14

7. Benitez M, Espinosa-Soto C, Padilla-Longoria P, Diaz J, Alvarez-Buylla ER. 2007. Equivalent genetic regulatory networks in different contexts recover contrasting spatial cell patterns that resemble those in *Arabidopsis* root and leaf epidermis: a dynamic model. *Int. J. Dev. Biol.* 51:139–55
8. Bhalla US, Iyengar R. 1999. Emergent properties of networks of biological signaling pathways. *Science* 283:381–87
9. Bintu L, Buchler NE, Garcia HG, Gerland U, Hwa T, et al. 2005. Transcriptional regulation by the numbers: models. *Curr. Opin. Genet. Dev.* 15:116–24
10. Bohn S, Andreotti B, Douady S, Munzinger J, Couder Y. 2002. Constitutive property of the local organization of leaf venation networks. *Phys. Rev. E.* 65:061914
11. Bolduc JE, Lewis LJ, Aubin CE, Geitmann A. 2006. Finite-element analysis of geometrical factors in microindentation of pollen tubes. *Biomech. Model Mechanobiol.* 5:227–36
12. Campilho A, Garcia B, Toorn HV, Wijk HV, Scheres B. 2006. Time-lapse analysis of stem-cell divisions in the *Arabidopsis thaliana* root meristem. *Plant J.* 48:619–27
13. Castle ES. 1937. Membrane tension and orientation of structure in the plant cell wall. *J. Cell. Comp. Physiol.* 10:113–21
14. Clark SE, Running MP, Meyerowitz EM. 1995. Clavata3 is a specific regulator of shoot and floral meristem development affecting the same processes as clavata1. *Development* 121:2057–67
15. Coen E, Rolland-Lagan AG, Matthews M, Bangham JA, Prusinkiewicz P. 2004. The genetics of geometry. *Proc. Natl. Acad. Sci USA* 101:4728–35
16. Corson F, Adda-Bedia M, Boudaoud A. 2009. In silico leaf venation networks: growth and reorganization driven by mechanical forces. *J. Theor. Biol.* 259:440–48
17. Corson F, Hamant O, Bohn S, Traas J, Boudaoud A, Couder Y. 2009. Turning a plant tissue into a living cell froth through isotropic growth. *Proc. Natl. Acad. Sci. USA* 106:8453–58
18. Cosentino Lagomarsino M, Tanase C, Vos JW, Emons AMC, Mulder BM, Dogterom M. 2007. Microtubule organization in three-dimensional confined geometries: evaluating the role of elasticity through a combined in vitro and modeling approach. *Biophys. J.* 92:1046–57
19. Cosgrove DJ. 2000. Expansive growth of plant cell walls. *Plant Physiol. Biochem.* 38:109–24
20. de Jong H. 2002. Modeling and simulation of genetic regulatory systems: a literature review. *J. Comput. Biol.* 9:67–103
21. de Reuille PB, Bohn-Courseau I, Godin C, Traas J. 2005. A protocol to analyze cellular dynamics during plant development. *Plant J.* 44:1045–53
22. de Reuille PB, Bohn-Courseau I, Ljung K, Morin H, Carraro N, et al. 2006. Computer simulations reveal properties of the cell-cell signaling network at the shoot apex in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* 103:1627–32
23. Denet B. 1996. Numerical simulation of cellular tip growth. *Phys. Rev. E. Stat. Phys. Plasmas Fluids Relat. Interdiscip. Topics* 53:986–92
24. Diaz J, Alvarez-Buylla ER. 2006. A model of the ethylene signaling pathway and its gene response in *Arabidopsis thaliana*: pathway cross-talk and noise-filtering properties. *Chaos* 16:023112
25. Dimitrov P, Zucker SW. 2006. A constant production hypothesis guides leaf venation patterning. *Proc. Natl. Acad. Sci. USA* 103:9363–68
26. Dumais J. 2007. Can mechanics control pattern formation in plants? *Curr. Opin. Plant Biol.* 10:58–62
27. Dumais J, Kwiatkowska D. 2002. Analysis of surface growth in shoot apices. *Plant J.* 31:229–41
28. Dumais J, Long SR, Shaw SL. 2004. The mechanics of surface expansion anisotropy in *Medicago truncatula* root hairs. *Plant Physiol.* 136:3266–75
29. Dumais J, Shaw SL, Steele CR, Long SR, Ray PM. 2006. An anisotropic-viscoplastic model of plant cell morphogenesis by tip growth. *Int. J. Dev. Biol.* 50:209–22
30. Dumais J, Steele CR. 2000. New evidence for the role of mechanical forces in the shoot apical meristem. *J. Plant Growth Regul.* 19:7–18
31. Dupuy L, Mackenzie J, Rudge T, Haseloff J. 2008. A system for modelling cell-cell interactions during plant morphogenesis. *Ann. Bot. (Lond)* 101:1255–65
32. Espinosa-Soto C, Padilla-Longoria P, Alvarez-Buylla ER. 2004. A gene regulatory network model for cell-fate determination during *Arabidopsis thaliana* flower development that is robust and recovers experimental gene expression profiles. *Plant Cell* 16:2923–39

33. Feijo JA, Cox G. 2001. Visualization of meiotic events in intact living anthers by means of two-photon microscopy. *Micron.* 32:679–84
34. Feugier FG, Iwasa Y. 2006. How canalization can make loops: a new model of reticulated leaf vascular pattern formation. *J. Theor. Biol.* 243:235–44
35. Forterre Y, Skotheim JM, Dumais J, Mahadevan L. 2005. How the Venus flytrap snaps. *Nature* 433:421–25
36. Fujita H, Mochizuki A. 2006. Pattern formation of leaf veins by the positive feedback regulation between auxin flow and auxin efflux carrier. *J. Theor. Biol.* 241:541–51
37. Galway ME, Masucci JD, Lloyd AM, Walbot V, Davis RW, Schiefelbein JW. 1994. The TTG gene is required to specify epidermal cell fate and cell patterning in the *Arabidopsis* root. *Dev. Biol.* 166:740–54
38. Geier F, Lohmann JU, Gerstung M, Maier AT, Timmer J, Fleck C. 2008. A quantitative and dynamic model for plant stem cell regulation. *PLoS One* 3:e3553
39. Gierer A, Meinhardt H. 1972. A theory of biological pattern formation. *Kybernetik*: 12(1):30–39
40. Glazier JA, Graner F. 1993. Simulation of the differential adhesion driven rearrangement of biological cells. *Phys. Rev. E. Stat. Phys. Plasmas Fluids Relat. Interdiscip. Topics* 47:2128–54
41. Goldsmith MH, Goldsmith TH, Martin MH. 1981. Mathematical analysis of the chemosmotic polar diffusion of auxin through plant tissues. *Proc. Natl. Acad. Sci. USA* 78:976–80
42. Gonzalez RC, Woods RE. 2008. *Digital image processing*. Upper Saddle River, NJ: Prentice Hall. xxii, 954 pp.
43. Gor V, Elowitz M, Bacarian T, Mjolsness E. 2005. *Tracking Cell Signals In Fluorescent Images*. Presented at Proc. 2005 IEEE Comput. Soc. Conf. Comput. Vision and Pattern Recogn., San Diego, CA
44. Gordon SP, Heisler MG, Reddy GV, Ohno C, Das P, Meyerowitz EM. 2007. Pattern formation during de novo assembly of the *Arabidopsis* shoot meristem. *Development* 134:3539–48
45. Goriely A, Neukirch S. 2006. Mechanics of climbing and attachment in twining plants. *Phys. Rev. Lett.* 97:184302
46. Grandjean O, Vernoux T, Laufs P, Belcram K, Mizukami Y, Traas J. 2004. In vivo analysis of cell division, cell growth, and differentiation at the shoot apical meristem in *Arabidopsis*. *Plant Cell* 16:74–87
47. Graner F, Glazier JA. 1992. Simulation of biological cell sorting using a two-dimensional extended Potts model. *Phys. Rev. Lett.* 69:2013–16
48. Grieneisen VA, Xu J, Maree AF, Hogeweg P, Scheres B. 2007. Auxin transport is sufficient to generate a maximum and gradient guiding root growth. *Nature* 449:1008–13
49. Guido NJ, Wang X, Adalsteinsson D, McMillen D, Hasty J, et al. 2006. A bottom-up approach to gene regulation. *Nature* 439:856–60
50. Hamant O, Heisler MG, Jonsson H, Krupinski P, Uyttewaal M, et al. 2008. Developmental patterning by mechanical signals in *Arabidopsis*. *Science* 322:1650–55
51. Hansen CD, Johnson CR. 2005. *The Visualization Handbook*. Amsterdam; Boston: Elsevier-Butterworth Heinemann. xvii, 962 pp.
52. Harrison CJ, Roeder AH, Meyerowitz EM, Langdale JA. 2009. Local cues and asymmetric cell divisions underpin body plan transitions in the moss *Physcomitrella patens*. *Curr. Biol.* 19:461–71
53. Haseloff J, Dormand EL, Brand AH. 1999. Live imaging with green fluorescent protein. *Methods Mol. Biol.* 122:241–59
54. Heisler MG, Ohno C, Das P, Sieber P, Reddy GV, et al. 2005. Patterns of auxin transport and gene expression during primordium development revealed by live imaging of the *Arabidopsis* inflorescence meristem. *Curr. Biol.* 15:1899–911
55. Hillier J, Makowski D, Andrieu B. 2005. Maximum likelihood inference and bootstrap methods for plant organ growth via multi-phase kinetic models and their application to maize. *Ann. Bot. (Lond)* 96:137–48
56. Isnard S, Cobb AR, Holbrook NM, Zwieniecki M, Dumais J. 2009. Tensioning the helix: a mechanism for force generation in twining plants. *Proc. Biol. Sci.* 276:2643–50
57. Jaqaman K, Loerke D, Mettlen M, Kuwata H, Grinstein S, et al. 2008. Robust single-particle tracking in live-cell time-lapse sequences. *Natl. Methods* 5:695–702
58. Jonsson H, Heisler M, Reddy GV, Agrawal V, Gor V, et al. 2005. Modeling the organization of the WUSCHEL expression domain in the shoot apical meristem. *Bioinformatics* 21:I232–40

59. Jonsson H, Heisler MG, Shapiro BE, Meyerowitz EM, Mjolsness E. 2006. An auxin-driven polarized transport model for phyllotaxis. *Proc. Natl. Acad. Sci. USA* 103:1633–38
60. Kaern M, Elston TC, Blake WJ, Collins JJ. 2005. Stochasticity in gene expression: from theories to phenotypes. *Nat. Rev. Genet.* 6:451–64
61. Katagiri F. 2003. Attacking complex problems with the power of systems biology. *Plant Physiol.* 132:417–19
62. Keller PJ, Schmidt AD, Wittbrodt J, Stelzer EH. 2008. Reconstruction of zebrafish early embryonic development by scanned light sheet microscopy. *Science* 322:1065–69
63. Kim HD, Shay T, O'Shea EK, Regev A. 2009. Transcriptional regulatory circuits: predicting numbers from alphabets. *Science* 325:429–32
64. Kroeger JH, Geitmann A, Grant M. 2008. Model for calcium dependent oscillatory growth in pollen tubes. *J. Theor. Biol.* 253:363–74
65. Kutschera U. 2008. The growing outer epidermal wall: design and physiological role of a composite structure. *Ann. Bot.* 101:615–21
66. Kwak SH, Shen R, Schiefelbein J. 2005. Positional signaling mediated by a receptor-like kinase in *Arabidopsis*. *Science* 307:1111–13
67. Kwiatkowska D, Dumais J. 2003. Growth and morphogenesis at the vegetative shoot apex of *Anagallis arvensis* L. *J. Exp. Bot.* 54:1585–95
68. Laguna MF, Bohn S, Jagla EA. 2008. The role of elastic stresses on leaf venation morphogenesis. *PLoS Computat. Biol.* 4:e1000055
69. Lee MM, Schiefelbein J. 2002. Cell pattern in the *Arabidopsis* root epidermis determined by lateral inhibition with feedback. *Plant Cell* 14:611–18
70. Li K, Miller ED, Chen M, Kanade T, Weiss LE, Campbell PG. 2008. Cell population tracking and lineage construction with spatiotemporal context. *Med. Image Anal.* 12:546–66
71. Li S, Assmann SM, Albert R. 2006. Predicting essential components of signal transduction networks: a dynamic model of guard cell abscisic acid signaling. *PLoS Biol.* 4:e312
72. Liu M, Roy-Chowdhury AK, Reddy GV. 2009. *Robust estimation of stem cell lineages using local graph matching*. Presented at IEEE Comput. Soc. Workshop Math. Methods in Biomed. Image Analysis, Miami Beach, FL
73. Locke JC, Kozma-Bognar L, Gould PD, Feher B, Kevei E, et al. 2006. Experimental validation of a predicted feedback loop in the multi-oscillator clock of *Arabidopsis thaliana*. *Mol. Syst. Biol.* 2:59
74. Lockhart JA. 1965. An analysis of irreversible plant cell elongation. *J. Theor. Biol.* 8:264–75
75. Long TA, Brady SM, Benfey PN. 2008. Systems approaches to identifying gene regulatory networks in plants. *Annu. Rev. Cell Dev. Biol.* 24:81–103
76. Mangan S, Alon U. 2003. Structure and function of the feed-forward loop network motif. *Proc. Natl. Acad. Sci. USA* 100:11980–85
77. Marcuzzo M, Quelhas P, Campilho A, Mendonca AM, Campilho A. 2008. *Automatic cell segmentation from confocal microscopy images of the* Arabidopsis *root*. Presented at IEEE Internat. Symp. Biomed. Imag.: From Nano to Macro, Paris
78. Martin MH, Goldsmith MH, Goldsmith TH. 1990. On polar auxin transport in plant cells. *J. Math Biol.* 28:197–223
79. Materi W, Wishart DS 2007. Computational systems biology in cancer: modeling methods and applications. *Gene Regul. Syst. Biol.*: 1:91–110
80. Mitchison GJ. 1981. The polar transport of auxin and vein patterns in plants. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 295:461–71
81. Mjolsness E, Sharp DH, Reinitz J. 1991. A connectionist model of development. *J. Theor. Biol.* 152:429–53
82. Mjolsness E, Yosiphon G. 2006. Stochastic process semantics for dynamical grammars. *Ann. Math. Artif. Intell.* 47:329–5
83. Modersitzki J. 2004. *Numerical Methods for Image Registration*. Oxford; New York: Oxford Univ. Press. x, 199 pp.
84. Mundermann L, Erasmus Y, Lane B, Coen E, Prusinkiewicz P. 2005. Quantitative modeling of *Arabidopsis* development. *Plant Physiol.* 139:960–68

85. Murray JD. 2002. *Mathematical Biology*. New York: Springer, 551 pp.
86. Newell AC, Shipman PD, Sun Z. 2008. Phyllotaxis: cooperation and competition between mechanical and biochemical processes. *J. Theor. Biol.* 251:421–39
87. Niklas KJ. 1977. Applications of finite-element analyses to problems in plant morphology. *Ann. Bot.* 41:133–53
88. Okada K, Ueda J, Komaki MK, Bell CJ, Shimura Y. 1991. Requirement of the auxin polar transport system in early stages of *Arabidopsis* floral bud formation. *Plant Cell* 3:677–84
89. Paradez A, Wright A, Ehrhardt DW. 2006. Microtubule cortical array organization and plant cell morphogenesis. *Curr. Opin. Plant. Biol.* 9:571–78
90. Paredez AR, Somerville CR, Ehrhardt DW. 2006. Visualization of cellulose synthase demonstrates functional association with microtubules. *Science* 312:1491–95
91. Pesch M, Hulskamp M. 2004. Creating a two-dimensional pattern de novo during *Arabidopsis* trichome and root hair initiation. *Curr. Opin. Genet. Dev.* 14:422–27
92. Pradal C, Dufour-Kowalski S, Boudon F, Fournier C, Godin C. 2008. OpenAlea: a visual programming and component-based software platform for plant modelling. *Funct. Plant Biol.* 35:751–60
93. Prusinkiewicz P, Coen E. 2007. Passing the El Greco test. *HFSP J.* 1:152–55
94. Prusinkiewicz P, Erasmus Y, Lane B, Harder LD, Coen E. 2007. Evolution and development of inflorescence architectures. *Science* 316:1452–56
95. Prusinkiewicz P, Lindenmayer A. 1990. *The Algorithmic Beauty of Plants*. New York: Springer-Verlag. xii, 228 pp.
96. Prusinkiewicz P, Rolland-Lagan AG. 2006. Modeling plant morphogenesis. *Curr. Opin. Plant Biol.* 9:83–88
97. Raj A, van Oudenaarden A. 2008. Nature, nurture, or chance: stochastic gene expression and its consequences. *Cell* 135:216–26
98. Reddy GV, Heisler MG, Ehrhardt DW, Meyerowitz EM. 2004. Real-time lineage analysis reveals oriented cell divisions associated with morphogenesis at the shoot apex of *Arabidopsis thaliana*. *Development* 131:4225–37
99. Reddy GV, Meyerowitz EM. 2005. Stem-cell homeostasis and growth dynamics can be uncoupled in the *Arabidopsis* shoot apex. *Science* 310:663–67
100. Reddy GV, Roy-Chowdhury A. 2009. Live-imaging and image processing of shoot apical meristems of *Arabidopsis thaliana*. *Methods Mol. Biol.* 553:305–16
101. Reinhardt D, Mandel T, Kuhlemeier C. 2000. Auxin regulates the initiation and radial position of plant lateral organs. *Plant Cell* 12:507–18
102. Reinhardt D, Pesce ER, Stieger P, Mandel T, Baltensperger K, et al. 2003. Regulation of phyllotaxis by polar auxin transport. *Nature* 426:255–60
103. Rolland-Lagan AG, Bangham JA, Coen E. 2003. Growth dynamics underlying petal shape and asymmetry. *Nature* 422:161–63
104. Rolland-Lagan AG, Coen E, Impey SJ, Bangham JA. 2005. A computational method for inferring growth parameters and shape changes during development based on clonal analysis. *J. Theor. Biol.* 232:157–77
105. Runions A, Fuhrer M, Lane B, Federl P, Rolland-Lagan AG, Prusinkiewicz P. 2005. Modeling and visualization of leaf venation patterns. *ACM Trans. Graph.* 24:702–11
106. Sachs T. 1981. The control of patterned differentiation of vascular tissues. *Adv. Bot. Res.* 9:151–262
107. Sahlin P, Soderberg B, Jonsson H. 2009. Regulated transport as a mechanism for pattern generation: capabilities for phyllotaxis and beyond. *J. Theor. Biol.* 258:60–70
108. Savage NS, Walker T, Wieckowski Y, Schiefelbein J, Dolan L, Monk NA. 2008. A mutual support mechanism through intercellular movement of CAPRICE and GLABRA3 can pattern the *Arabidopsis* root epidermis. *PLoS Biol.* 6:e235
109. Savageau MA. 1969. Biochemical systems analysis. II. The steady-state solutions for an n-pool system using a power-law approximation. *J. Theor. Biol.* 25:370–79
110. Scarpella E, Marcos D, Friml J, Berleth T. 2006. Control of leaf vascular patterning by polar auxin transport. *Genes Dev.* 20:1015–27
111. Scheres B. 2001. Plant cell identity. the role of position and lineage. *Plant Physiol.* 125:112–14

112. Schnittger A, Folkers U, Schwab B, Jurgens G, Hulskamp M. 1999. Generation of a spacing pattern: the role of triptychon in trichome patterning in *Arabidopsis*. *Plant Cell* 11:1105–16
113. Schopfer P. 2006. Biomechanics of plant growth. *Am. J. Bot.* 93:1415–25
114. Segel IH. 1975. *Enzyme Kinetics: Behavior and Analysis of Rapid Equilibrium and Steady State Enzyme Systems*. New York: Wiley. xxii, 957 pp.
115. Shapiro BE. 2008. Image Analysis Software for Computational Morphodynamics. **http://computational-morphodynamics.net**
116. Shapiro BE, Mjolsness E. 2001. *Developmental simulations with cellerator*. Presented at 2nd Intern. Conf. Syst. Biol., Pasadena, CA
117. Shipman PD, Newell AC. 2005. Polygonal planforms and phyllotaxis on plants. *J. Theor. Biol.* 236:154–97
118. Smith RS, Bayer EM. 2009. Auxin transport-feedback models of patterning in plants. *Plant Cell Environ.* 32:1258–71
119. Smith RS, Guyomarc'h S, Mandel T, Reinhardt D, Kuhlemeier C, Prusinkiewicz P. 2006. A plausible model of phyllotaxis. *Proc. Natl. Acad. Sci. USA* 103:1301–6
120. Strogatz SH. 1994. *Nonlinear Dynamics and Chaos : With Applications to Physics, Biology, Chemistry, and Engineering*. Reading, MA: Addison-Wesley xi, 498 pp.
121. Swain PS, Elowitz MB, Siggia ED. 2002. Intrinsic and extrinsic contributions to stochasticity in gene expression. *Proc. Natl. Acad. Sci. USA* 99:12795–800
122. Szallasi Z, Stelling J, Periwal V. 2006. *System Modeling in Cell Biology: From Concepts to Nuts and Bolts*. Cambridge, MA: MIT Press. xiv, 448 pp.
123. Truernit E, Bauby H, Dubreucq B, Grandjean O, Runions J, et al. 2008. High-resolution whole-mount imaging of three-dimensional tissue organization and gene expression enables the study of phloem development and structure in *Arabidopsis*. *Plant Cell* 20:1494–503
124. Turing AM. 1952. The chemical basis of morphogenesis. *Philos. Transac. R. Soc. Lond. B Biol. Sci.* 237:37–72
125. Tyson JJ, Chen KC, Novak B. 2003. Sniffers, buzzers, toggles and blinkers: dynamics of regulatory and signaling pathways in the cell. *Curr. Opin. Cell Biol.* 15:221–31
126. Veytsman BA, Cosgrove DJ. 1998. A model of cell wall expansion based on thermodynamics of polymer networks. *Biophys. J.* 75:2240–50
127. Walter A, Silk WK, Schurr U. 2009. Environmental effects on spatial and temporal patterns of leaf and root growth. *Annu. Rev. Plant Biol.* 60:279–304
128. Wasteneys GO, Ambrose JC. 2009. Spatial organization of plant cortical microtubules: close encounters of the 2D kind. *Trends Cell Biol.* 19:62–71
129. Watanabe T, Hanan JS, Room PM, Hasegawa T, Nakagawa H, Takahashi W. 2005. Rice morphogenesis and plant architecture: measurement, specification and the reconstruction of structural development by 3D architectural modelling. *Ann. Bot.* (*Lond*) 95:1131–43
130. Wolpert DH, Macready WG. 1997. No free lunch theorems for optimization. *IEEE Transac. Evol. Computat.* 1:67
131. Wymer CL, Wymer SA, Cosgrove DJ, Cyr RJ. 1996. Plant cell growth responds to external forces and the response requires intact microtubules. *Plant Physiol.* 110:425–30
132. Yu Z, Holst M, Cheng Y, McCammon JA. 2008. High-fidelity geometric modeling of biomedical applications. *Finite Elem. Anal. Des.* 44:715–23
133. Yu ZY, Holst MJ, Cheng YH, McCammon JA. 2008. Feature-preserving adaptive mesh generation for molecular shape modeling and simulation. *J. Mol. Graphics Model.* 26:1370–80
134. Zeilinger MN, Farre EM, Taylor SR, Kay SA, Doyle FJ, 3rd. 2006. A novel computational model of the circadian clock in *Arabidopsis* that incorporates PRR7 and PRR9. *Mol. Syst. Biol.* 2:58
135. Zhang Y, Bajaj C, Sohn B. 2005. 3D finite element meshing from imaging data. *Comput. Methods Appl. Mech. Eng.* 194:5083–106

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