REVIEW

Multiscale Systems Analysis of Root Growth and Development: Modeling Beyond the Network and Cellular Scales

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Over recent decades, we have gained detailed knowledge of many processes involved in root growth and development. However, with this knowledge come increasing complexity and an increasing need for mechanistic modeling to understand how those individual processes interact. One major challenge is in relating genotypes to phenotypes, requiring us to move beyond the network and cellular scales, to use multiscale modeling to predict emergent dynamics at the tissue and organ levels. In this review, we highlight recent developments in multiscale modeling, illustrating how these are generating new mechanistic insights into the regulation of root growth and development. We consider how these models are motivating new biological data analysis and explore directions for future research. This modeling progress will be crucial as we move from a qualitative to an increasingly quantitative understanding of root biology, generating predictive tools that accelerate the development of improved crop varieties.

INTRODUCTION

Understanding the mechanisms that control root growth and development is essential for compelling scientific, economic, and environmental reasons. Food security represents a pressing global issue. Crop production must double by 2050 to keep pace with global population growth (OECD-FAO Agricultural Outlook 2012 to 2021; http://www.oecd.org/site/oecd-faoagriculturaloutlook/). This target is even more challenging given the impact of climate change on water availability and the drive to make agriculture more sustainable by reducing fertilizer inputs. The development of crops with improved water and nutrient uptake efficiency will help address these challenges. Root architecture critically influences nutrient and water uptake efficiency: Rooting depth impacts nitrogen and water acquisition as both leach deep into the soil (Foulkes et al., 2009), whereas the angle of root growth determines whether roots explore the topsoil where phosphate accumulates (Ge et al., 2000).

Over recent decades, molecular genetic approaches in *Arabidopsis thaliana* have identified many of the key signals and transduction components that control root development (Benfey et al., 2010), leading the field to move beyond studying the functions of individual gene products to focus instead on determining the relationships between multiple components that

compose the regulatory pathways that control root biology. However, these signals and transduction components operate within pathways that often function in a nonlinear fashion, integrating negative and positive feedback loops (Middleton et al., 2012). Computational and mathematical modeling approaches are set to become much more important as our knowledge of pathways becomes increasingly detailed and their network behavior and outputs less intuitive. Attempts to model these signals and their transduction pathways need to go beyond the network scale and capture the cellular, tissue, and organ scales, considering how regulatory networks function across multiple root cells and tissues and how they regulate tissue- and organ-scale biomechanical properties to control growth and development (summarized in Figure 1). Moreover, roots grow and develop within soil, arguably the most complex environment on the planet, where they are subject to an array of abiotic and biotic signals and stresses. Given these factors, it is often very difficult to predict the impact that a particular gene or genes will have on a root trait or entire system. Multiscale models, which consider behaviors at several or all of the subcellular, cell, tissue, organ, and whole-organism scales, enable us to capture this complexity and generate new mechanistic insights into the regulation of root growth and development (Chickarmane et al., 2010).

Three points should be made at the outset to clarify the content of this review. First, we focus primarily on multiscale models of primary and lateral roots that span from network to organ scales (directing readers interested in the modeling of
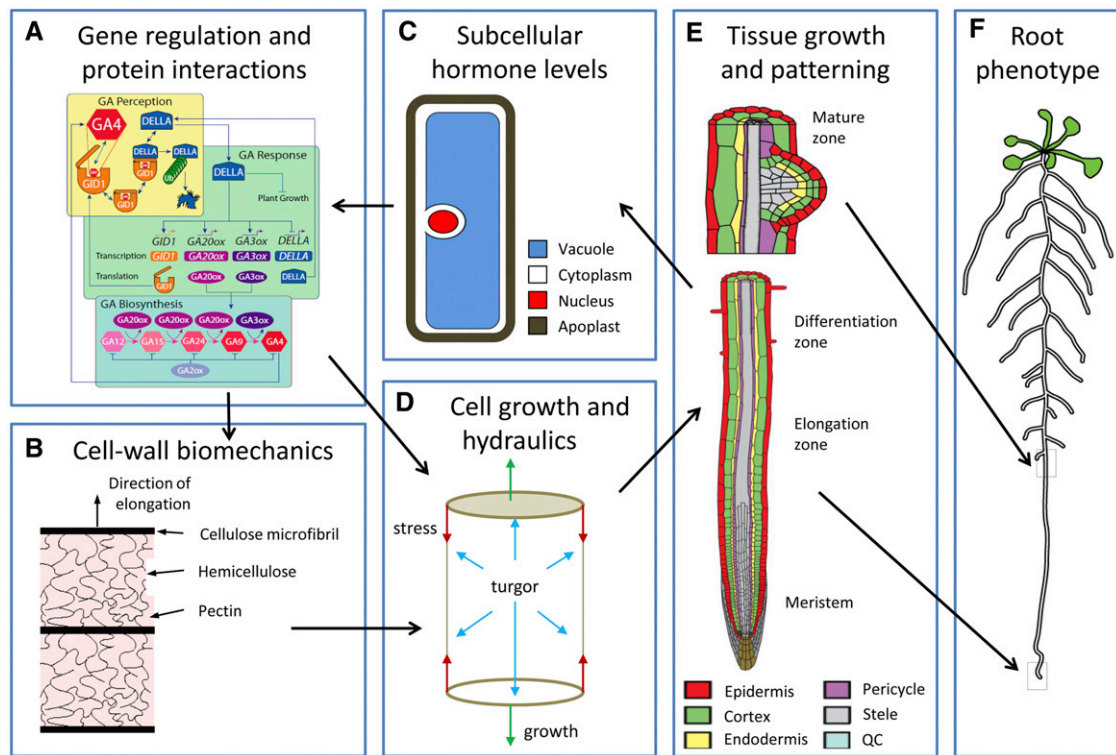
Figure 1. Key Interacting Network/Cell/Tissue-Scale Processes during Root Growth and Development.

Predicting root growth and development **(F)** requires understanding of gene regulation and protein interactions **(A)** and how these affect the cell wall biomechanics **(B)** and hydraulics **(D)** that determine cell growth. Cell growth in turn causes growth and patterning on the tissue scale **(E)**, which feeds back on the subcellular hormone levels **(C)** and, hence, gene regulation. QC, quiescent center. [*Panel A reprinted from Middleton et al. (2012), Figure 1*].

entire root systems to the excellent reviews of Dupuy et al. [2010a] and Draye et al. [2010]). Second, we are concerned here exclusively with mechanistic modeling, whereby the mathematical models seek explicitly to capture the requisite biological phenomena, rather than purely phenomenological approaches in which the models aim to reproduce observations without necessarily reflecting the mechanisms responsible. Third, it is important to emphasize that the models in question make no attempt to capture the full complexity of real biological systems and their results should in consequence not be overinterpreted, no matter how realistic and appealing the visualizations of computational simulations may appear. The adage "as simple as possible but no simpler" applies here: The models are of necessity abstractions of biological reality and seek properly to reflect, and hence enhance the understanding of, the key biological processes of interest without obscuring these processes by including illusory levels of complexity.

This review first introduces several key concepts and techniques employed in multiscale models. We then describe the key elements needed to develop multiscale models (Figure 1). We describe communication between and within cells by signals and components of gene regulatory networks and highlight the importance of integrating cell wall mechanics and hydraulic processes (elements that are largely overlooked in root models

to date). Next, we describe examples of multiscale models that contain two or more of these key elements. Finally, we consider how new techniques are capturing multiscale data for multiscale models and explore directions for future research, discussing the need to embed plant root models into descriptions of the whole plant and the plant-soil-atmosphere system.

MULTISCALE MODELING TECHNIQUES: A BRIEF OVERVIEW

Mechanistic models typically comprise mathematical or computational descriptions of a biological process, which can be solved (either numerically or analytically) to predict the behavior of the biological process for a given set of model assumptions. Thus, modeling forces us to think in a mechanistic manner; it enables us to evaluate whether a hypothesized set of mechanisms produces observed dynamics, allows us to assess the problems with our intuition when predictions and observations do not agree, and provides a framework to investigate the relative roles of different biological processes. Multiscale models consider behaviors on two or more scales, ranging from the subcellular up to whole-organism scales and beyond, thereby requiring the integration of systems-biology formulations with

more traditional physiological descriptions. For the purposes of this review, the spatial scales in question (and the approaches used to model them) can be characterized as follows: (1) sub-cellular: capturing gene regulatory, signaling, and metabolic networks, typically using Boolean, ordinary differential equations (ODEs), or (when low copy numbers are important) stochastic models (e.g., Karlebach and Shamir, 2008) (using different representations for the amount of substances in each cell and different types of rules for their evolution in time); (2) cell scale: capturing transport and mechanical interactions between neighboring cells, typically on the basis of individual-based models in which the individuals are single cells or cellular compartments; (3) tissue and organ scales: describing growth, transport, and deformation on the macroscale, traditionally adopting continuum (mainly partial differential) equations that do not explicitly subdivide a tissue into discrete cells.

While the demarcation between these scales is often blurred, this subdivision leads naturally to bottom-up, middle-out, and top-down viewpoints, respectively. Although the bottom-up (network) scale has seen explosive growth in recent years due to the impact of new genomic technologies (Bassel et al., 2012), the most natural and appropriate approach is arguably middle-out (Noble, 2006), in which models are constructed starting with the level at which we have the most information. Cells provide a natural level of organization for an organism, so cell-based models in which individual cells can be endowed with considerable internal machinery (representing the subcellular networks) and the interactions of numerous individual cells lead to the (emergent) tissue-scale properties are an attractive approach, naturally incorporating heterogeneity in cellular properties; this is the viewpoint that will receive most emphasis in subsequent sections.

A range of different approaches have been used to develop cell-based models of plant roots. The main differences between these models lie in how the individual cells are represented geometrically and how the physical and chemical interactions between cells are treated. Cellular Potts (or Glazier-Graner-Hogeweg) models represent cells as collections of voxels (volumetric elements, typically cuboids) that undergo growth through stochastic relabeling rules (Graner and Glazier, 1992; Glazier and Graner, 1993). Frameworks of this type were initially developed to treat animal cell growth, for which the very flexible representation of cell shape is an advantage. Such models have been used to describe auxin transport and growth in plant roots (Grieneisen et al., 2007; Laskowski et al., 2008). However, the underlying formulation of Potts models leads to difficulties in accurately capturing viscous deformations, and additional constraints are required to prevent cells sliding past each other.

Vertex-based models (Weliky and Oster, 1990; Nagai and Honda, 2001), in which cells are described by polygons (in two dimensions) or polyhedra (in three dimensions) are an appealing approach for modeling plant tissues, as the relatively regular shape of cells (with near planar interfaces between neighboring cells) is well approximated, and symplastic growth is naturally enforced by the sharing of vertices between neighboring cells. Complications that occur in the application of such models to animal tissues, such as the topological transitions when cells change their neighbors, occur only in a limited set of situations,

such as cell separation during lateral root emergence (Swarup et al., 2008). Such models have been developed for plant tissues by a number of authors (Rudge and Haseloff, 2005; Jönsson et al., 2006; Dupuy et al., 2008; Hamant et al., 2008; Stoma et al., 2008; Merks et al., 2011).

As these and other multicellular simulation techniques have become more well established, software frameworks such as CompuCell 3D (Cickovski et al., 2005), CellModeller (Dupuy et al., 2007), CHASTE (Pitt-Francis et al., 2009), OpenAlea (Pradal et al., 2008), Organism (http://dev.thep.lu.se/organism/), and VirtualLeaf (Merks et al., 2011) have been produced that make it possible to develop multicellular simulations more efficiently, building on existing collections of code.

In contrast with these computationally intensive approaches, analytical (i.e., mathematical) multiscale methods are increasingly being used to study plant dynamics. These methods aim to relate tissue-level descriptions to processes that occur at a finer scale, for example, showing how a quantity such as the tissue-level hormone velocity depends on the detailed cell-level transport processes (Band and King, 2012), and thus can be used to derive simpler descriptions of complex models, essentially moving from a cell-based description to a continuum approximation. While such tissue-level (continuum) descriptions have traditionally been used in top-down approaches, these descriptions have had essentially phenomenological components: Relating the quantities that appear within them to the cellular and subcellular properties is a crucial and challenging aspect of multiscale mechanistic modeling. Systematic mathematical procedures (associated with homogenization theory other multiple-scale asymptotic methods—these being theories associated with analytical approaches that exploit disparate length or time scales to derive effective equations for the large-scale dynamics that incorporate the effects of smaller scale processes) exist for achieving such integration in some contexts, but significant further developments are required if they are fully to realize their potential (Bensoussan et al., 1978; Mei and Vernescu, 2010). Significant subtleties arise in their application, notably in accounting for non-negligible cumulative effects that may arise from processes that do not appear significant on the finer scales. That such developments are nevertheless well worth pursuing is apparent not only from the greater generality inherent in such continuum formulations, but also because the resulting models are computationally tractable over scales on which it would be impractical to account separately for all the individual cells. Hybrid models that couple tissue-level descriptions for certain processes with cell-based descriptions for others also have a significant role to play.

To date, the majority of multiscale studies of plant roots have considered the model plant *Arabidopsis*. The *Arabidopsis* primary root is ideally suited to perform multiscale modeling studies given its simple radial organization, which consists of a series of concentric layers of epidermal, cortical, endodermal, and pericycle tissues surrounding the central vascular tissues (Figure 1). This is matched by the apical-basal developmental gradient of cells transiting the meristem, elongation, and differentiation zones as they first divide, then expand and ultimately adopt specialized cell fates (Figure 1E). Much of our discussion below will therefore focus on this model species.

KEY ELEMENTS IN MULTISCALE MODELS

Communication between Cells

Communication between cells is essential to ensure that cell growth and development are coordinated to form a well-structured, patterned tissue. In plants, such communication often involves mobile transcription factors or hormones, which move between adjacent cells and interact with their signaling networks (biomechanical interactions also play a crucial role; see below). In many cases, the dynamics depend on complex regulation at the cellular and subcellular scales; understanding how this regulation produces the dynamic tissue-scale distribution is often non-intuitive, making multiscale modeling an essential part of the research process. For example, patterning through the mobile transcription factor SHORTROOT (SHR) was analyzed by Azpeitia et al. (2010) who consider the stem cell niche in the root tip and modeled auxin-dependent regulation of the PLETHORA family of transcription factors, along with the mobile protein SHR and its partner SCARECROW. The model suggested additional interactions that would provide a plausible mechanism for the maintenance of the stem cell niche and highlighted the gaps in the experimental understanding of the underlying networks.

Multiscale models have addressed the transport of several hormones, including auxin (Swarup et al., 2005; Grieneisen et al., 2007), gibberellin (Band et al., 2012a), and cytokinin (Chavarria-Krauser et al., 2005). These models of gibberellin and cytokinin have supposed passive diffusion between adjacent cells, taking the simplest assumption, which may require revision as biological knowledge increases (Cedzich et al., 2008). By contrast, auxin has been shown to move through root tissues in a polar manner due to the spatial distributions of specialized auxin influx (i.e., AUX1 or LAX) and/or efflux proteins (i.e., PIN) present on the cell membranes (Kramer and Bennett, 2006). This mobile signal plays a key role in patterning root tissues and, in particular, maintaining the apical meristem (Grieneisen et al., 2007), regulating the gravitropic response (Swarup et al., 2005) and promoting the initiation of lateral roots (Laskowski et al., 2008) and of root hairs (Jones et al., 2009).

The dynamics of hormone and mobile transcription factors can be described by cell-based models consisting of coupled systems of ODEs for the concentrations in a collection of well-mixed compartments; some such models represent each cell as a single compartment, whereas others subdivide the tissue further, considering subcellular or cell wall compartments. These ODEs depend on the tissue geometry, cell sizes, and cell–cell connectivity: The fluxes between adjacent compartments depend on the length of the dividing boundary and the fluxes change the concentrations in a manner dependent on the compartments' volumes. For a given set of parameter values, these ODEs can be simulated to predict the distributions and fluxes. This approach has been used in numerous auxin transport models. For example, Swarup et al. (2005) developed a cell-based three-dimensional model of auxin transport in the three outer layers in the root elongation zone (Figure 2A). The model found that AUX1 was essential for producing the shootward flux
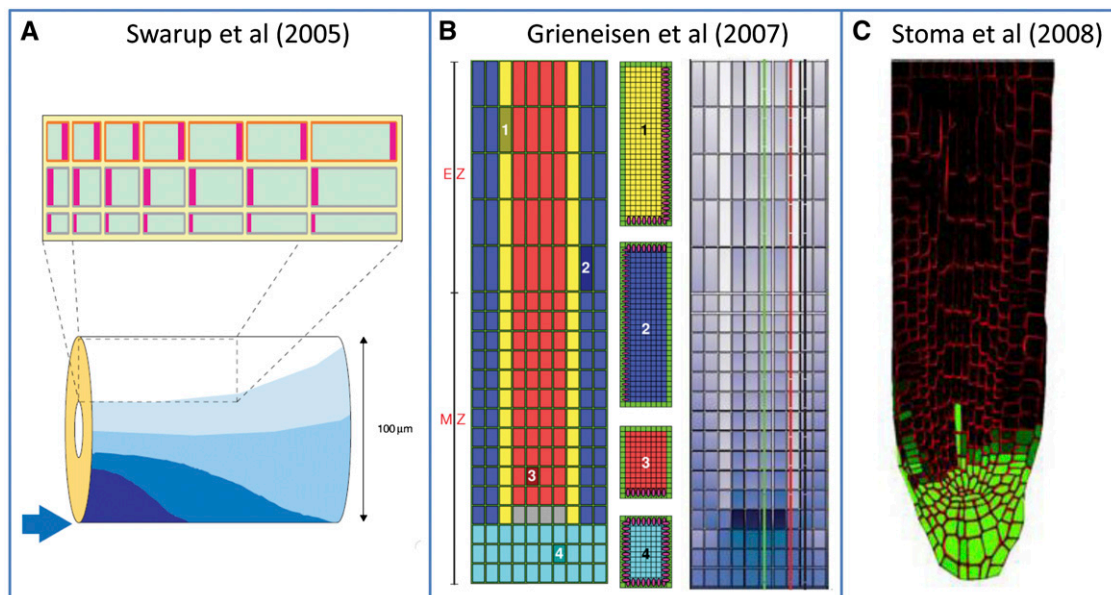

Figure 2. Recent Multiscale Auxin Transport Models.

(A) A three-dimensional model from Swarup et al. (2005) predicted the auxin distribution in root elongation zone tissues following a gravitropic stimulus. [*Adapted by permission from Macmillan Publishers Ltd: Nature Cell Biol. Swarup et al. (2005), Figure 2.*]

(B) A two-dimensional model from Grieneisen et al. (2007) predicted the auxin distribution in the growing root tip, using idealized cell geometries. [*Adapted by permission from Macmillan Publishers Ltd: Nature. Grieneisen et al. (2007), Figure 1.*]

(C) A two-dimensional model from Stoma et al. (2008) predicted the auxin distribution in the root tip using cell geometries extracted from confocal images. EZ, elongation zone; MZ, meristematic zone. [*Adapted from Stoma et al. (2008), Figure 10.*]

required for gravitropic bending. This model was later extended by prescribing AUX1 only to specific epidermal cell files to understand their role in initiating root hairs (Jones et al., 2009). A related two-dimensional model of the root tip was developed by Grieneisen et al. (2007) (Figure 2B): This captured the reversed fountain, incorporating rootward auxin flow through the root center and reflux at the quiescent center, demonstrating how the PIN distribution can create an auxin maximum at the quiescent center and how the predicted auxin gradient can pattern the growth dynamics. Similar approaches to study auxin dynamics have been used in models of the root tip (Stoma et al., 2008; Mironova et al., 2010, 2012), lateral root initiation (Laskowski et al., 2008), and root nodule infection (Perrine Walker et al., 2010). Computational studies have also considered the stochasticity of auxin transport dynamics, simulating the movement of individual auxin molecules (rather than describing the dynamics with ODEs); stochastic simulations were shown (in the context of a simple model) to agree with ODE solutions and provided the expected variation in the auxin distribution (Twycross et al., 2010).

While many cell-based auxin transport models have considered a prescribed fixed carrier distribution, these techniques have also been used to study the emergent dynamics due to auxin's regulation of its own carriers. To date, the mechanisms behind this regulation are not established; studies have investigated the implications of different models (reviewed in Smith and Bayer, 2009). Stoma et al. (2008) reported that the reversed fountain pattern in the root tip could self-organize with a flux-based mechanism, whereby the level of PIN on a membrane depends on the auxin flux (Figure 2C), whereas Mironova et al. (2010, 2012) proposed a reflected-flow mechanism with auxin-dependent production and degradation of the PIN proteins within each cell (and the PIN positions prescribed). Considering the differentiation zone, Laskowski et al. (2008) demonstrated that the positive feedback created by auxin upregulating its influx carriers could lead to auxin maxima that trigger lateral root formation. Other models of PIN regulation have recently been proposed, considering, for example, the roles of intercellular gradients (Kramer, 2009), mechanical stress (Heisler et al., 2010), or an extracellular receptor (Wabnik et al., 2010); these are likely to be incorporated into root models in the near future.

Due to the regular geometry and patterning of plant root tissue, analyzing auxin transport also naturally lends itself to asymptotic methods, relating spatially discrete cell-based models to their continuum tissue-scale description. By relating each compartment to its spatial position, one can simplify a system of hundreds of ODEs governing a cell-based model to a few partial differential equations describing the evolving hormone concentration in space and time. Continuum approaches have been explored by Band and King (2012) to analyze how cell-scale PIN and AUX1 distributions affect the auxin velocity through root outer layers. Similar homogenization techniques can also capture auxin transport through subcellular compartments, as implemented by Chavarria-Krauser and Ptashnyk (2010) in analyzing auxin transport through a uniform array of cells. These analytical techniques also enable one to assess the relative importance of different cell-scale processes, for example, determining the parameter regimes in which cell wall diffusion affects the tissue-scale auxin distribution (Band and King, 2012).

Gene Transcription and Protein Interactions

Translating spatio-temporal variations in levels of signals, such as hormones, into differences in cellular behaviors requires regulatory networks that incorporate gene transcription and translation and protein–protein interactions (Muraro et al., 2011). Much progress has been made in developing mathematical models of plant hormone response pathways that have increased understanding of network dynamics.

A gene regulatory model has been constructed for the nuclear auxin response pathway (Middleton et al., 2010). This signaling network contains auxin/indole-3-acetic acid (Aux/IAA) family proteins that repress the activity of Auxin Response Factors (ARFs) transcription factors through forming heterodimers (Mockaitis and Estelle, 2008). Auxin leads to increased degradation of Aux/IAA, thereby preventing the repression of ARF-bound loci and increasing the transcription of auxin-responsive genes, including many members of the Aux/IAA family. Middleton et al. (2010) considered a single representative species for each of the ARF and Aux/IAA protein families and identified the importance of the turnover rates of Aux/IAA protein and mRNA for the dynamical behavior of the model. Bridge et al. (2012) extended the model of part of Middleton et al. (2010) to include two different Aux/IAAs and ARFs, finding that the resulting network could exhibit bistability. In a similar vein, Vernoux et al. (2011) included both ARF activators and repressors and homodimerization of Aux/IAAs and used their model to understand spatial differences in auxin sensitivity and the buffering effect of part of the auxin response network in the shoot apical meristem. A model of part of the auxin response network has also demonstrated the potential of using the new Aux/IAA-based fluorescent reporter DII-VENUS (Brunoud et al., 2012) to quantify auxin concentrations. The DII-VENUS fluorescent protein is constitutively expressed and degraded in the presence of auxin in a TIR1-dependent manner (Brunoud et al., 2012). By developing and parameterizing a model of DII-VENUS degradation, Band et al. (2012b) quantified the dynamic relationship between auxin and DII-VENUS. Using the resulting parameterized model to interpret DII-VENUS measurements, the authors showed that the dynamics of auxin redistribution in root tissues following a gravitropic stimulus exhibited striking gradient on–gradient off behavior (Band et al., 2012b). The high temporal resolution provided by this integrated systems approach helped validate key predictions made by the Choldny-Went hypothesis, including that auxin gradient formation (within 2 to 3 min of a gravity stimulus) happens prior to root bending (first detected 10 to 20 min after the gravity stimulus).

Gene regulatory network models have also been developed for other hormones, including abscisic acid (ABA; Dupeux et al., 2011), gibberellin (Middleton et al., 2012), and cytokinin (Muraro et al., 2011). Dupeux et al. (2011) developed a stochastic model for the ABA response network that revealed the importance of oligomerization of receptor proteins in permitting this signal to generate responses over a wide range of concentrations and suggested that this was a possible mechanism for cells to control their sensitivity to ABA. Middleton et al. (2012) modeled the gibberellin response network, revealing that conformational changes in the gibberellin receptor control the time scale of the response and demonstrating the importance of feedback loops in the response network.

Many hormone response pathways interact through shared components (Nemhauser et al., 2006). For instance, gibberellin (Willige et al., 2011) and cytokinin (Marhavý et al., 2011) have been described as regulating PIN carrier abundance. Similarly, cytokinin has been noted to promote transcription of Aux/IAAs and thus reduce the PIN expression, while auxin is thought to promote the transcription of a cytokinin signaling repressor (Müller and Sheen, 2008). Given the complexity of these interactions, mathematical models have an essential role to play in understanding the effects of perturbing these networks and how multiple signals are integrated to control development and growth. For example, Muraro et al. (2011) recently revealed how cytokinin may control the oscillatory behavior of the auxin response network. Similarly, Liu et al. (2010) modeled the crosstalk between auxin, cytokinin, and ethylene signaling and examined the role of the *POLARIS* (*PLS*) gene. The model explained the increased cytokinin levels in the root apex of the *pls* mutant and predicted that PLS regulates auxin biosynthesis. Using this model, the role of PLS in controlling the effect of ethylene on the auxin level in the root tip was explained, with PLS regulating the effect of ethylene on both auxin biosynthesis and transport. Sankar et al. (2011) considered crosstalk between auxin and brassinosteroid signaling. Their model was used to explore possible roles for the auxin-inducible *BREVIS RADIX* (*BRX*) gene; in order to explain mutant phenotypes, the model suggested that BRX has a positive effect on brassinosteroid synthesis and a positive effect on auxin-responsive genes. This positive effect was predicted to be ARF dependent, and experimental studies confirmed an interaction between BRX and MONOPTOROS/ARF5 (Scacchi et al., 2010).

Mechanics

The downstream targets of many regulatory networks are components that alter the biomechanical properties of root tissues (Swarup et al., 2008; Péret et al., 2012), thereby modifying organ growth and development. Determining how these network outputs influence observed root phenotypes requires a detailed understanding of cell wall biomechanics and water transport in root tissues.

Multiscale mathematical and computational models allow the exploration of how biophysical processes operate within the constraints imposed by the laws of physics and root cell and tissue geometry. During organ elongation (driven by anisotropic cell expansion) and bending (arising via differential elongation), a root must overcome potentially large external forces (e.g., soil compaction) and respond to a variety of environmental cues (such as gravity and chemical gradients). These are achieved through the root's elaborate multiscale architecture combined with its capacity to regulate its mechanical properties in space and time.

The individual cell provides a natural starting point in building an in silico model of root growth, from the biomechanical as well as the biochemical point of view. Cell rigidity can be achieved by a tensegrity mechanism (Ingber, 1993), whereby a pressurized vacuole is confined within a stiff cell wall, with tension in the wall balancing internal turgor. Strong intercellular adhesion extends this rigidity to the whole tissue. The cell wall is reinforced with cellulose microfibrils that are laid down in organized patterns, endowing the wall with anisotropic properties and allowing the isotropic turgor force to drive elongation in a preferred direction (Baskin, 2005; Cosgrove, 2005). The root thereby behaves like a hydraulic pump, translating osmotic potential into a force that can drive the organ into soil.

The cell wall has multiple constituents that can be tuned individually to alter its mechanical properties. Its pectin matrix is a gel in which deesterified polygalacturonan molecules are cross-linked by calcium ions (Cosgrove, 2005). Hemicellulose polymers cross-link the cellulose microfibrils that are embedded in the pectin matrix. Different enzymes target these distinct components: Pectin methyl esterase promotes calcium cross-linking (Proseus and Boyer, 2006), enzymes of the XTH family cut hemicellulose cross-links (Rose et al. 2002), and expansin cleaves hemicellulose cross-links from microfibrils (McQueen-Mason et al., 1992). Mathematical models that capture the contribution of each individual component to the mechanical properties of the composite structure can be used to infer how a particular enzyme influences a cell wall; such models can then be extended in a multiscale context to predict the overall effect of an enzyme at tissue and organ levels.

Root growth occurs primarily by targeted softening of primary cell walls. This is accompanied by synthesis and delivery of new wall material as the cells expand. Mathematical models describing growth must therefore couple descriptions of metabolic processes to constitutive equations (stress/strain or stress/strain-rate relationships) that describe the material properties of cells or tissues (Boudaoud, 2010). A root cell may respond elastically on short time scales but exhibit viscous behavior over longer time scales. Large irreversible deformations are often taken to occur only once the driving turgor pressure exceeds a critical yield stress. The most widely used mathematical description of plant cell growth is provided by the Lockhart equation (Lockhart, 1965): Assuming water fluxes are not rate limiting, this equation relates elongation rate to the turgor pressure that drives unidirectional cell expansion. The Lockhart equation can be applied at multiple length scales (from segments of cell wall up to whole tissues), serving both as a constitutive relation and as an empirical model. The associated material parameters (extensibility and yield) can be measured for large samples (such as algal *Chara* cells), although direct measurements on typical root cells are scarce. However, while being appealingly simple, the Lockhart equation does not contain the level of detail required in continuum mechanical models to describe bodies undergoing bending or twisting deformations in two or three coordinate directions. Even for simple unidirectional expansion, numerous refinements to the Lockhart equation have been suggested (Ortega, 1990; Passioura and Fry, 1992; Dyson and Jensen, 2010; Pietruszka, 2011) that capture specialized features of cell and tissue expansion.

Different approaches have been taken in understanding the mechanical properties of a multicomponent cell wall, where chemical interactions play a major role in determining the rheology. The WallGen model (Kha et al., 2010) simulates directly the interactions between large numbers of cellulose microfibrils and hemicellulose cross-links. Veytsman and Cosgrove (1998)

used thermodynamic principles to relate the stiffness and yield stress to properties of the microfibril/hemicellulose network. In a study of growing pollen tubes, Rojas et al. (2011) modeled a network of calcium cross-links to show how the extensibility of the pectin gel relates to its stiffness and cross-link dissociation rate; their model connects chemical and deposition kinetics to wall expansion rates. Building on the conceptual framework of Passioura and Fry (1992), Dyson et al. (2012) used transient viscoelastic network theory to describe the evolution of hemicellulose cross-links in an elongating cell wall, recovering yielding behavior reminiscent of a Lockhart model. These recent models shed new light on the mechanical effects of enzyme action, either of enzymes acting on hemicellulose cross-links (XTH and expansin; Dyson et al., 2012) or on the matrix (pectin methyl esterase; Rojas et al., 2011).

The orientation of cellulose microfibrils comes to the fore in determining the shape of individual expanding cells, allowing classical engineering theories for fiber-reinforced composites to be exploited (Huang et al., 2012). Adapting one such theory to a thin-walled cell, Dyson and Jensen (2010) derived from fundamental principles a form of the Lockhart equation that incorporates fiber reorientation; they adopted the multinet hypothesis (Green et al., 1971), which proposes that once deposited on the inner surface of the cell wall, fibrils reorient passively as the cell elongates, thereby resisting further cell extension. There is evidence supporting the concept of passive fiber reorientation (Anderson et al., 2010); there is also a growing body of evidence supporting the concept that the stress environment in the cell influences the angle of fibril deposition, mediated by the underlying microtubule network (Hamant et al., 2008; Hamant and Traas, 2010).

Since mechanical properties of plant cells are dominated by their walls, vertex-based models provide a natural framework for computational simulation of tissue-scale mechanics (Rudge and Haseloff, 2005; Jönsson et al., 2006; Dupuy et al., 2008; Hamant et al., 2008). The Lockhart equation (or one of its variants) is a common building block in such models, as it describes microstructural processes in a simple form. Many of these models have incorporated anisotropy in the mechanical properties of cells (e.g., by allowing the elastic properties of cell walls to depend upon their direction relative to the microtubule direction in each cell) (Hamant et al., 2008). Another feature common to many models is to treat the cell walls as bending beams (Dupuy et al., 2008), for instance, in the investigation of the effect of depolymerizing microtubules in a growing plant root (Corson et al., 2009).

At the tissue scale, the root can be treated as a continuum: Here, homogenization approaches can again be applied in averaging micromechanical models to derive suitable constitutive laws; typically, these recapitulate the visco-plastic behavior embodied in the Lockhart equation but are then amenable to traditional engineering analysis, for example, using finite-element methods (Huang et al. 2012). In both vertex-based and continuum approaches, the important contribution of multiscale methods is to relate tissue-level properties systematically to processes occurring at the level of the cell wall and to enable mechanics to be coupled to the biological processes driving root growth and development.

Hydraulics and Nutrient Transport

Most recent biomechanical models focus on the role of the cell wall, neglecting spatial variations in cell turgor and supposing that water fluxes are not rate-limiting (i.e., water transport is fast compared with the time scale of growth). However, root hydraulics, a subject of historic physiological studies that is key to understanding plant growth, cannot be overlooked. Roots uptake water and nutrients from the soil, requiring them to pass radially through concentric cell layers to reach the root vasculature; close to the root tip, cells also require water to grow, drawing it from both the soil and the vasculature (Boyer et al., 2010).

Water and nutrients move through plant tissue through several parallel, coupled pathways: within the apoplast (intracellular pathway), between cell cytoplasms through plasmadesmata (symplastic pathway), and across a cell's plasma membrane, the permeability of which is regulated by aquaporins (Steudle, 2000). Fluxes along these pathways can vary spatially and temporally: For example, the Casparian strip is a physical barrier that greatly reduces the porosity and permeability of the endodermal apoplast, whereas aquaporin levels are under genetic regulation (Javot and Maurel, 2002). Within and between each pathway, water flows are driven by gradients in hydrostatic pressure and osmotic potential, whereas nutrient transport is driven by concentration gradients (i.e., diffusion), convection (when dissolved in water), and active transport (for example, via ion channels). As nutrients move through tissues, changing osmotic potentials affect water transport, resulting in a highly coupled system.

Multiscale studies have modeled water and nutrient transport through nongrowing root tissue. Models predicting the spatial variations in water fluxes, turgor pressures, and cell volumes have been investigated analytically by a number of authors, deriving tissue-scale models from cell-scale descriptions (reviewed in Molz, 1981). Supposing water transport to occur only from cell to cell and taking the osmotic potentials between cells to be equal, Philip (1958a, 1958b) demonstrated that water transport could be described by a diffusion equation, with a diffusion coefficient depending on the cell-scale parameters. Related expressions for the diffusion coefficient were deduced taking into account a diffusible solute (Molz and Hornberger, 1973), the cell wall pathway (Molz and Ikenberry, 1974), and the symplastic pathway (Molz, 1976). Subsequent authors considered the role of water fluxes in sustaining steady growth, investigating the gradients in water potential that drive water and solutes into the growing cells. Molz (1978) derived and solved one-dimensional equations for the radial water fluxes required to sustain axial hypocotyl growth. Related continuum equations have been used to determine the water potential distribution in a three-dimensional model of the root elongation zone, neglecting the presence of the vasculature and assuming axial growth (Silk and Wagner, 1980); this model was extended by Wiegers et al. (2009) to include water fluxes from the phloem.

An alternative approach to modeling water and nutrient transport has been to neglect the cellular structure of the tissue altogether and consider water flows between compartments

representing collections of cells, capturing the resistance of many cell membranes, by prescribing properties to a single effective membrane. A number of studies (cf. the review of Murphy, 2000) have taken this approach to understand the interplay between steady-state water and solute fluxes in nongrowing tissue: Such models explain the observed non-linear relationship between flux and applied pressure.

A compartmental approach has recently been adopted by Péret et al. (2012) to investigate how aquaporins regulate lateral root emergence. In *Arabidopsis*, lateral root primordia (LRP) originate from pericycle cells located deep within the parental root and have to emerge through endodermal, corti-cal, and epidermal tissues (see schematic in Figure 1E). Re-cent studies have highlighted the importance of the localized auxin signal originating from the tip of the LRP to induce specific physiological responses in overlaying tissues (Swarup et al., 2008; Péret et al., 2012). Biomechanical properties tar-geted by auxin include changes in aquaporin spatial expres-sion patterns to modify water fluxes. Péret et al. (2012) include a mathematical model describing how aquaporin-dependent tissue hydraulics could affect the timing of lateral root deve-lopment. In the model, water fluxes are coupled to primordium expansion, with the LRP and overlaying tissue represented as two fluid compartments. The dividing primordium cells have a prescribed increasing osmotic potential. The model predicts the resulting water fluxes and pressure dynamics and shows how these drive primordium expansion. The model predicts that auxin's aquaporin repression promotes lateral root emergence and explains the experimentally observed delay in LRP emergence for both aquaporin loss-of-function and over-expression lines.

EXAMPLES OF MULTISCALE MODELS

Relatively few examples of multiscale models that combine two or more of the elements detailed above have been described to date for roots or other plants organs (for examples in shoot tissues, see Murray et al., 2012). The following examples were selected to illustrate the power of developing multiscale models to probe the mechanisms underlying complex, nonlinear bi-ological processes in roots.

Coupling Root Growth with Auxin Regulation

Key to predicting the relationship between genotype and phe-notype is linking the spatio-temporal concentrations of regulators, such as auxin, with cell growth and division. While a mechanistic understanding of the effect of auxin on growth is currently lacking, several existing examples employ multiscale approaches, in-corporating phenomenological models to describe this missing step.

Grieneisen et al. (2007) proposed that the specification of the root's developmental zones is controlled by a gradient of auxin, supposing that cell division occurs at high hormone concen-trations and cell elongation at lower concentrations. Due to the predicted auxin distribution (Figure 2B), their model simulates cell's division and growth dynamics in the meristem and elon-gation zone, capturing the gradual expansion of the meristem

over the first 8 d after germination and the reduction in meristem size after root excision. Chavarria-Krauser et al. (2005) modeled the growth dynamics at the root tip, supposing that the ratio between auxin and cytokinin governs the production and deg-radation of a remodeling enzyme, which in turn regulates cell growth and division. Considering root developmental re-sponses, Lucas et al. (2008b), in predicting lateral root initiation, considered a pool of auxin at the root tip that is consumed by root gravitropic bending and/or emergence. Using such a phe-nomenological model between auxin and root development, the authors were able to accurately predict experimentally observed perturbations in lateral root initiation for the *aux1* root gravitropic and *lax3* lateral root emergence mutants.

Coupled models of auxin transport and growth have often been significantly simplified by considering the time scales involved: Since auxin moves through plant tissue at ∼1 cm/h (Kramer et al., 2011), auxin dynamics typically occur much faster than cell growth and division, enabling one to treat auxin distributions as quasi-steady when simulating cell growth and division (i.e., simulating the auxin dynamics until an equilibrium distribution is achieved, before taking one step of cell growth/division) (Stoma et al., 2008), or, conversely, allowing one to use static geometries when ana-lyzing auxin dynamics (Band and King, 2012).

Gibberellin Dilution Can Explain Root Growth Dynamics

The hormone gibberellin has been shown to control root growth, affecting both cell division within the root meristem (Ubeda-Tomás et al., 2009) and cell growth within the elon-gation zone (Ubeda-Tomás et al., 2008). Band et al. (2012a) recently developed a multiscale model of gibberellin dynamics in the *Arabidopsis* root elongation zone, prescribing cell growth (using experimental measurements) and simulating gibberellin dilution, diffusion of gibberellin between cellular compartments, and the response network through which gibberellin degrades the growth-repressing DELLA proteins (describing the dynamics with a system of ODEs). The model revealed that, as cells pass through the elongation zone, dilution creates a declining gib-berellin concentration, leading to spatial gradients in the levels of downstream mRNAs and proteins. In particular, the levels of growth-repressing DELLA proteins are predicted to be high at the end of the elongation zone, consistent with the reduction in cell growth at this location. The study also considered the dynamics in plants treated with paclobutrazol (an inhibitor of gibberellin biosynthesis) and plants with mutations in the gib-berellin biosynthesis and signaling pathways; these cases re-vealed that the growth rates appear to reflect the fold change in DELLA as cells traverse the elongation zone. Furthermore, the model provided new insights into the normal phenotype ex-hibited in the *ga1-3 gai-t6 rga-24* triple mutant. The model demonstrated that the effect of the *ga1-3* mutation in reducing gibberellin biosynthesis (leading to higher functional DELLA) can counteract the effect of the *gai-t6 rga-24* mutation in reducing the translation of functional DELLA; if these two processes are suitably balanced, the levels of functional DELLA are similar to the wild type, explaining why the triple mutant exhibits normal cell elongation. In summary, by assimilating a range of data and knowledge, the model deduced the dominant effect of

gibberellin dilution on the emergent DELLA distribution, providing new insights into gibberellin's growth regulation.

Epidermal Root Cell Patterning

Epidermal cells in the *Arabidopsis* root are patterned into alternating files of trichoblasts (bearing root hairs) and atrichoblasts (lacking root hairs), where trichoblasts form from epidermal cells overlying two cortical cell files. This patterning is regulated by mobile activator proteins GALABRA3/ENHANCER OF GLABRA3 (GL3/EGL3), which form an activator complex with TRANS-PARENT TESTA GALABRA1 and WEREWOLF (WER), and the mobile inhibitor protein CAPRICE (CPC). Savage et al. (2008) formulated a stochastic Boolean model, embedded into a circle of cells, with the levels of CPC and GL3/EGL3 depending on the rate of transcription in the neighboring cells. This was used to explore potential network structures, comparing WER self-activation and CPC inhibition via competition for GL3/EGL3 with constitutive WER transcription and CPC directly inhibiting WER. While both potential network structures replicated the patterning in wild-type roots, the latter was found to best explain mutant phenotypes, in particular *cpc*. Benítez et al. (2008) explored the same problem using a (three-state) deterministic model within a two-dimensional array of cells; although they also found that WER autoregulation was not required for pattern formation in wild-type roots, it proved necessary to explain some mutant phenotypes in the two-dimensional geometry. This two-dimensional model was also used to explore the effects of cell shape, with cell elongation acting to stabilize patterns. Although experiments of Savage et al. (2008) provided evidence that WER autoregulation is not important for the early patterning of epidermal cells, local self-activation has been found to occur at later stages (Kwak and Schiefelbein, 2008; Kang et al., 2009).

MULTISCALE DATA FOR MULTISCALE MODELS

As modeling technologies become both richer and more widely applied, demand naturally increases for data with which to build, parameterize, and validate the resulting models. These data must be quantitative, as accurate as possible, and span at least the range of scales considered by the model at hand. Although the initial data required may be clearly identifiable, the complete data set needed by a particular modeling project can be difficult to predict. Techniques that allow large amounts of raw data to be analyzed to provide a range of quantitative measures, reducing subjectivity and increasing repeatability by minimizing human involvement, are the ideal. Digital images and automatic image analysis methods provide a particularly rich source of data for multiscale root modeling.

Though automatic image analysis provides a wide variety of tools, most root image analysis methods rely on either segmentation or visual tracking. Image segmentation seeks to divide the pixels (or voxels) making up the input image into a set of distinct regions, each corresponding to a separate biological object. Once achieved, this allows quantitative measurements of the properties of those objects (e.g., area and length) to be made. Recovery of kinematic data requires some feature of the root to be identified in each of a sequence of images and its relative position or shape to be described; this recovery can be limited by complexity of the object being tracked and the degree to which its movement can be predicted. Methods have been proposed that track root features at a variety of scales: individual cells (Marcuzzo et al., 2008b; Roberts et al., 2010), tissue patches (van der Weele et al., 2003; Sethuraman et al., 2012), root tips (Campilho et al., 2006), and whole (primary; Figure 3A) roots (French et al., 2009).

Subcellular-Scale Data

Effective modeling of processes such as hormone transport and protein interactions requires quantitative data on a subcellular scale. Specifically, data are needed on the amount of a given target within a single voxel or other image region. Protein quantification is a challenging problem, as many factors affect the relationship between the number of molecules of a fluorescent reporter in a particular region and the image values that result (Pawley, 2000). Though true quantification remains a challenge, relative measures are possible (Band et al., 2012b). Protein colocalization is a related problem: The challenge here is to quantify the extent to which two signals, in separate color channels of a confocal laser microscope image (Stephens and Allan, 2003), are correlated. Current approaches range from application of simple statistical measures (French et al., 2008) to schemes that simultaneously estimate the degree of colocalization and identify image regions in which it is considered significant (Costes et al., 2004). Reliable colocalization, however, remains an active research topic (Bolte and Cordelières, 2006).

Cellular- and Tissue-Scale Data

Confocal laser scanning microscopy is the most widely used imaging modality at the cellular level. Confocal images, however, generally provide higher quality data on outer cell layers; deeper into the tissue the laser source is attenuated and the microscope's response becomes weaker. During segmentation, this can result in cells being merged into a single region as boundaries become unclear. It can also lead to oversegmentation (single cells being represented by many small regions) as image noise disrupts the segmentation process.

A number of authors have recently adapted the classical watershed algorithm (Vincent and Soille, 1991) to address the oversegmentation problem: Marcuzzo et al. (2008a) improved the results by training a classifier to recognize pairs of regions that should be merged; Dupuy et al. (2010b) initialized the watershed segmentation process manually, by placing a marker in each cell; and Federici et al. (2012) performed segmentation by simulating the inflation of a flexible balloon within each cell (a technique that performs well, even on quite poor quality images). While high-quality images, and so high-quality segmentation and geometry, can be obtained reliably across the sample by fixing the specimen before imaging (a destructive process), similar quality segmentation was recently achieved for a live tissue using a watershed algorithm in conjunction with confocal stacks acquired from multiple viewpoints (Fernandez et al., 2010).

Using an alternative approach, Sethuraman et al. (2012) and Pound et al. (2012) represented the plant root as a cell network (Figure 3B), rather than as a set of neighboring individuals, and achieved segmentation by optimizing the fit of a flexible network of cell walls to the input image. More accurate (two-dimensional)

geometry is provided than by techniques based on single cells, although laser attenuation and noise can still prevent extraction of a complete cell network.

Organ-Scale Data

A variety of methods have been proposed to extract root system architecture descriptions from standard digital images of plant roots. These can now provide length and curvature data (Figure 3A) on younger, simpler root architectures with minimal human intervention (French et al., 2009; Naeem et al., 2011). Older plants, in which large numbers of fine roots touch and cross each other, are more challenging, and usually require higher levels of user involvement (Armengaud et al., 2009; Le Bot et al., 2010; Lobet et al., 2011). Light-based imaging technology is widely available, and multicamera techniques can support three-dimensional reconstruction (Iyer-Pascuzzi et al., 2010; Clark et al., 2011), but it is limited to laboratory-based growth environments. Although rhizotrons (buried, transparent camera housings) allow limited access to roots in soil, there is some concern that the presence of the rhizotron may influence root development (Neumann et al., 2009). X-ray microcomputed tomography, however, is now being used to image plant roots grown in soil noninvasively (Mooney et al., 2012). Computed tomography (CT) has the potential to provide rich three-dimensional descriptions of the geometry of older plants, and

Mairhofer et al. (2012) have recently described a segmentation method capable of separating roots from soil in three-dimensional x-ray micro CT data. Magnetic resonance imaging and positron emission tomography methods capable of directly imaging plant function (e.g., water transport or carbon allocation) are also under investigation (Jahnke et al., 2009).

FUTURE CHALLENGES FOR MULTISCALE ROOT MODELS

This review has highlighted how multiscale mathematical and computational models are proving invaluable tools with which to generate new mechanistic insights into the regulation of root growth and development. These models provide a strong foundation to analyze root growth, although in order to remain relevant, they will need to be continually adapted to incorporate new biological findings and reflect current hypotheses. Furthermore, we are only at the beginning of this new research area, and several challenging issues are flagged below if we are to proceed further.

Integrating Models (Conceptual and Technological Challenges)

The pioneering models highlighted in this review have in general been designed and validated independently of one another, raising important issues relating to interoperability. To proceed
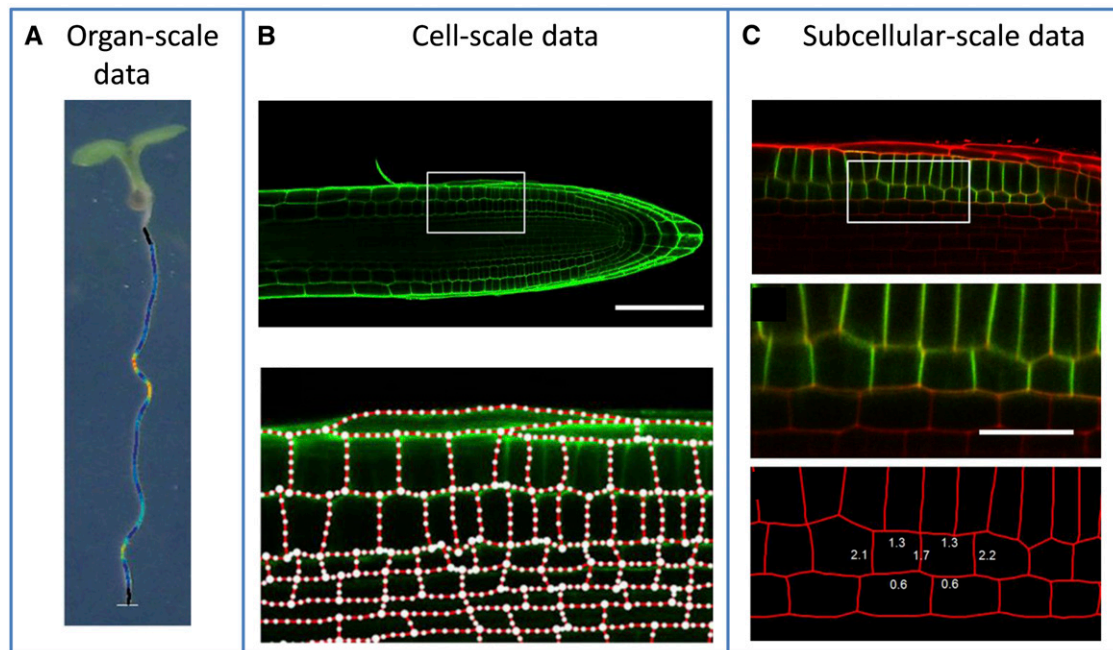

Figure 3. Extracting Quantitative Data from Digital Images at Multiple Scales.

(A) Color images of plated *Arabidopsis* seedlings provide length, curvature, and growth data (French et al., 2009). [*Reprinted from French et al. (2012), Figure 3.*]

(B) Segmentation of confocal laser scanning microscopy images provides realistic tissue and cell geometries (Pound et al., 2012). [*Reprinted from Pound et al. (2012), Figure 1.*]

(C) Protein concentration and localization data can be recovered from confocal microscope images (red channel, propidium iodide; green channel, PIN2–green fluorescent protein) by first extracting cell-scale descriptions (Pound et al., 2012). [*Reprinted from Pound et al. (2012), Figure 3.*]

further in the investigation of root system development, a new level of complexity needs be addressed, and interactions between processes operating at different scales must be studied and modeled. For this, various models must be assembled into unified and consistent frameworks. This integration operation is made complex for a number of conceptual and technological reasons. (1) The basic models may operate at different spatial resolutions or in different dimensions (one, two, or three dimensions). For example, one may want to couple a two-dimensional mechanical model of tissue expressed at subcellular resolution with a two-dimensional model of auxin transport designed at cellular resolution. This may require homogenizing the spatial resolution so that the cell geometries are updated throughout time by the mechanical model, and in some cases, redesigning the models to adapt them to the chosen unifying spatial resolution. (2) Models may also have different temporal resolutions. For example, when coupling mechanical and genetic processes, additional assumptions must be made concerning the typical time unit at which both processes must be simulated. In this case, one classical assumption is that cell growth rates are much slower than biochemical processes; consequently, cell size and shape can be considered constant, while chemical simulations are performed. In other cases, a hierarchy between the different typical time units cannot be defined and the interaction between corresponding processes thus needs to be explicitly modeled. (3) Not only can spatial and temporal scales be different, but the mathematical formalisms may also be. Combining different types of mathematical model (Boolean, piecewise-affine, stochastic, ODE, and partial differential equation) poses an additional theoretical challenge. First models integrating both mechanistic and stochastic models have been designed at organismal level on aerial systems (Costes et al., 2008). Taking into account stochasticity in root modeling is now in reach (Lucas et al., 2008a) and may prove essential to address the full complexity of the branching systems. (4) When coupling models, we are also faced with technological issues. Such combination of models will represent the realization of large amounts of theoretical and experimental effort, and it is important that these are freely available to the community through either shared data sets, open-source model formats, such as SBML (Hucka et al., 2003) or CellML (Cuellar et al., 2003), or common modeling software platforms, for example, VV (Prusinkiewicz et al., 2004), OpenAlea (Pradal et al., 2008), or MorphoGraphX (http://www.sybit.net/software/MorphoGraphX).

Truly Multiscale Data

Multiscale modeling brings new challenges for the image analyst by demanding multiscale data from a single image set. CELLSET (Pound et al., 2012), for example, extracts measurements at the tissue (cell network), cell (size and area), and molecular (protein concentration and localization) levels (Figures 3B and 3C) from confocal microscopy images. Though recovery of such complex data sets is challenging, the interactions between scales may actually simplify the problem. In CELLSET, for example, cell-level descriptions are used to constrain, and so ease, the search for subcellular objects. However, few image sets are rich enough to allow this level of data recovery; attention must instead be directed toward registering and integrating data obtained from different image types.

Toward a Digital Plant and Beyond

In the longer term, developing a mechanistic model of a whole plant (recently referred to as a digital plant model) represents a logical next step. Indeed, given the exchange of water, nutrients, and signals between root and shoot organs, developing a virtual root or shoot model in isolation of each other could be considered naïve in the longer term. To date, models of diverse root system subprocesses have been developed at different scales. Compared with initial approaches in systems biology, most of these models make explicit use of spatial information. Such spatial information represents different aspects of realistic root structures and can take the form of a continuous medium, a branching structure of connected elements (e.g., root meristems), a multicellular population, or a set of interacting subcellular compartments. By integrating progressively more functional aspects into these realistic representations, modelers are creating models known as functional-structural plant models (FSPMs; Sievänen et al., 2000; Godin et al., 2005). FSPMs therefore provide a promising platform with which to create a digital plant model.

Compared with many previous FSPMs developed on aerial parts (Vos et al., 2010) and on root systems (Bidel et al., 2000; Pagès et al., 2004), recent FSPMs also integrate gene regulation and signaling as a new dimension in the analysis of development (Prusinkiewicz et al., 2007; Han et al., 2010). Through the combined modeling of genetic networks, physiological processes, and spatial interaction between components, a new generation of FSPMs is being developed that opens the way to building digital versions of real plants (Coen et al., 2004; Cui et al., 2010) and testing biological or agronomical hypotheses in silico (Stoma et al., 2008; Perrine-Walker et al., 2010). Ultimately, plant performance is measured by breeders at the population scale, rather than the individual. Hence, mechanistic multiscale models need to be developed that bridge the remaining physical scale between the plant and field to ensure that we are able to relate genotype to phenotype and engineer crop traits.

Integrating Sensitivity to Environmental Parameters into Multiscale Models

Phenotype represents the output of the interaction between genotype and environment. Almost all of the models described in this review study intrinsic regulatory processes during root development. This provides a good foundation for describing the basic developmental mechanisms; however, to account for the plasticity of root system development, models must also integrate environmental factors. A major future challenge will be to develop mixed genetic-ecophysiological models that bridge the gap between genetic and environmental regulation (Roose and Schnepf, 2008; Draye et al., 2010).

Environmental factors that should be captured in rhizosphere models include physical properties of the soil, water availability, nitrogen distributions, macro/microelements, mycorrhiza/nodulation, and competition or interaction with other root systems. How

these factors affect the development of roots, and the variability of their architecture, will be key questions to address. One key challenge facing the development of multiscale models for the rhizosphere is data integration. Soil structure is traditionally assessed by imaging and then physically removing successive layers of soil, while root washing remains the most common method of recovering descriptions of roots grown in soil. As both are destructive, construction of combined root and soil data sets requires integration of measurements obtained from different samples. X-ray micro-CT has the potential to provide rich image data sets from single samples, presenting concurrent measurements of root (Mairhofer et al., 2012) and soil (Mooney et al., 2012) parameters that will provide novel insights, for example, into how pore space depends on local root architecture. Nevertheless, new methodologies to assay other rhizosphere properties, such as nutrient distribution, are also required. Armed with such information, we will be well placed to develop realistic multiscale root models that bridge the genotype-phenotype gap and form predictive tools when optimizing crop root architectures for soil types and nutrient regimes.

AUTHOR CONTRIBUTIONS

All authors contributed to writing the article.

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