CellPress
REVIEWS

Review

In Silico Roots: Room for Growth

Jacob Pieter Rutten[1] and Kirsten ten Tusscher[1,*]

Computational models are invaluable tools for understanding the hormonal and genetic control of root development. Thus far, models have focused on the crucial roles that auxin transport and metabolism play in determining the auxin signaling gradient that controls the root meristem. Other hormones such as cytokinins, gibberellins, and ethylene have predominantly been considered as modulators of auxin dynamics, but their underlying patterning mechanisms are currently unresolved. In addition, the effects of cell- and tissue-level growth dynamics, which induce dilution and displacement of signaling molecules, have remained unexplored. Elucidating these additional mechanisms will be essential to unravel how root growth is patterned in a robust and self-organized manner. Models incorporating growth will thus be crucial in unraveling the underlying logic of root developmental decision making.

Modeling Root Growth and Development

In the past two decades computational models have come to play an increasingly important role in deciphering the complex patterning processes that underlie plant development. Models allow incorporation of processes occurring at widely different spatial and temporal scales, enabling investigation of the causal relations between, and emergent properties, of processes that experimentally can often only be studied in isolation [1]. Second, in models one can systematically add, remove, or change variables and their interactions, allowing identification of the core mechanism underlying a biological patterning process. Finally, even if models fail to simulate the pattern of interest, they are a powerful tool to determine the nature of the missing pieces of the puzzle [2,3].

A particularly intensive research area in plant biology focuses on understanding the processes controlling root growth and development. At the tip of the roots, protected by a root cap and the gravity-sensing columella cells, reside the quiescent center (QC) cells surrounded by a stem cell niche (SCN) containing stem cells for each of the root cell-type lineages [4]. The QC and SCN undergo infrequent divisions to replenish the more shootward meristem as well as the tipward-lying columella. In the meristem, cells undergo rapid divisions, thereby producing the raw material for future root growth, while in the above lying elongation zone vacuolar swelling combined with cell-wall remodeling drives the rapid cellular expansion that generates actual root length growth [5,6]. Finally, in the differentiation zone, terminal differentiation of cells into their respective cell types takes place, generating new vascular transport tissues as well as new epidermal, cortical, and endodermal protective and uptake tissues (Figure 1, Key Figure; rightmost panel). Tight control of root zonation dynamics is essential to ensure maintenance of the QC and SCN to secure a persistent meristem, and hence future growth, while at the same time optimizing current growth to environmental conditions.

Root zonation is controlled by a highly complex interaction network of hormones, genes, peptides, and other signaling molecules. Center stage is occupied by a gradient of auxin that has its maximum in the QC and tapers off along the meristem (Figure 1, middle), and which is

Highlights

Computational models incorporating different spatial and temporal scales are an important tool in unraveling the complex patterning processes underlying root growth and development.

Models have succeeded in unraveling the mechanisms underlying the root-tip auxin gradient that patterns root meristems. At the same time, they have highlighted how a lack in knowledge currently prohibits a complete understanding of the antagonistic cytokinin domain in the control of elongation and differentiation.

Incorporating cell growth, division, and expansion into models is of crucial importance. First, by inducing dilution and displacement of signaling molecules, growth dynamics feeds back on the patterning network that controls it. In addition, explicitly considering that individual cells traverse the different root zones is likely to reveal important clues to root growth patterning.

[1]Computational Developmental Biology Group, Faculty of Science, Utrecht University, Utrecht, The Netherlands

*Correspondence:
k.h.w.j.tentusscher@uu.nl
(K. ten Tusscher).

involved in promoting stemness and sustaining division [7–9]. Auxin is antagonized by cytokinin (CK), which inhibits division and promotes elongation and differentiation, and whose signaling domain resides shootward of the meristem [10–13]. The meristem size and growth rate arising from this auxin–CK antagonism is further influenced by other hormones such as ethylene [14], gibberellin (GA) [12,15], abscisic acid (ABA) [16], and brassinosteroids [17]. However, it remains unclear to what extent the currently uncovered interactions are necessary and sufficient to explain the spatial patterning of hormones and developmental zones and what is the underlying logic of these networks. As an example, it appears that auxin may both induce [18,19] and repress [20,21] CK biosynthesis, but how these processes are separated in space, time, and under different conditions – and how this is functionally relevant – remains incompletely understood. In addition, how the architecture of the regulatory network enables **self-organized** (see Glossary) and robust patterning of root meristems, but at the same time allows flexible adjustment of root growth to environmental conditions, remains to be clarified.

The current review focuses on modeling studies investigating root growth and zonation, explaining their basic building blocks, incremental evolution, and the lessons they have taught us. In addition, we point out open questions and the type of model developments that will be necessary to answer these questions. Ultimately, a complete, mechanistic understanding of the checks and balances involved in controlling root growth and development should enable future targeted breeding of designer crops with desired root growth traits.

The Building Blocks of Root Growth Models

Modeling Root Anatomy

Root zonation models are typically multiscale, describing the dynamics at both the single-cell, local tissue, and overall root-tip levels. Although simplified 1D spatial root models have been developed, typically simulating a single vascular cell strand [22] (Figure 2A), we mostly focus on models describing a 2D longitudinal cross-section of the root tip that incorporate the different cell types of the root (Figure 2B–D). Cell types may differ with respect to cell width, as well as in the types, levels, and polarity patterns of auxin-exporting **PIN proteins** and auxin-importing **AUX/LAX proteins**. Early models incorporated a simplified, square-shaped layout of root topology [7,23–25] (Figure 2B), whereas more recent models incorporate an anatomically correct root-tip layout, either idealized [10] (Figure 2C) or from digitized microscopy images [26–29] (Figure 2D). Importantly, the realism of root-tip shape has been shown to have significant consequences for simulated auxin patterns [30] and transport [2] (Figure 2).

Models also often incorporate the distinct developmental zones of the root. Implemented differences typically entail cell length increasing from the meristem, through the elongation zone, and into the differentiation zone. In addition, some models incorporate differences in terms of hormone transport, hormone activity, or gene expression. Finally, the locations of zone boundaries may either be superimposed or are defined in terms of the hormone or protein gradients that evolve from the patterning dynamics.

Modeling Gene Expression and Hormonal Signaling

To model how networks of interactions between gene expression and hormone signaling control root zonation patterning, the dynamical behavior of the relevant factors and their responses to one another need to be modeled. Proteins often incorporated into root zonation models are: (i) hormone transporters (e.g., the auxin-exporting PINs), (ii) hormone biosynthesis and degradation enzymes (e.g., IPTs involved in CK biosynthesis), and (iii) hormone receptors and downstream factors (e.g., GID and DELLA for GA). In addition, some models also simulate the dynamics of more downstream transcription factors such as the QC-specific WOX5 that

Glossary

AUX/LAX proteins: active auxin-importing proteins that are embedded in the plasma membranes of specific cells, allowing them to take up more auxin versus only passive uptake across the membrane.

Chemiosmotic theory of auxin transport: auxin is a weak acid that, inside cells with pH levels of ~7, exists mostly in its deprotonated form, preventing passive export out of the cell. By contrast, in cell walls, owing to the lower local pH level (5.5), auxin is mostly protonated, enabling passive import into cells. As a consequence, auxin transport is dominated by active auxin export.

Ordinary differential equations (ODEs): equations that express the current rate of change of a set of variables at their current value. They have only one independent variable, usually time.

Partial differential equations (PDEs): have more than one independent variable, such as time and space.

PIN proteins: active auxin-exporting proteins that are embedded in the plasma membrane, typically with a cell type-specific polar orientation giving rise to oriented auxin transport.

Reflected flow: PIN1-mediated flow of auxin down towards, but not entirely to, the root tip arises from the bell-shaped dependence of PIN1 levels on auxin concentration that enables self-organized patterning of an auxin maximum in the absence of a full-blown reflux loop, and hence is complementary to the reflux loop mechanism, controls outside of the systemReflux looproot level. While the reflux loop enables maintenance of the auxin maximum in absence of shoot derived auxin, the reflected flow enables re-establishment of an auxin maximum after root tip excision.

Reflux loop: the root-level PIN polarization pattern that gives rise to auxin flux down into the root tip in the inner root tissues, combined with the upward- and inward-oriented flux in the outer tissues that causes gradual recycling of auxin back into the downward-flowing tissues, thus forming a loop.

control cell fate and behavior. Gene expression and intracellular signaling dynamics are typically modeled using so-called **ordinary differential equations** (ODEs) that describe the cell-level processes promoting or reducing the factor of interest, thereby also modeling the links of the interaction network (Box 1).

To model the dynamics of mobile signals such as hormones, one must also consider transport processes. In most models, passive and active transport into and out of cells is considered [26–29,31], while in others intracompartmental diffusion is also modeled to allow for subcellular and sub-wall concentration differences [7,10,23] (Box 1). Unfortunately, thus far it has not been investigated what consequences these different modeling choices may have for simulation outcomes (see Outstanding Questions).

Root zonation models typically involve a substantial number of parameters, for many of which values are often not easily available through experimentation. As a consequence, model development may benefit from applying efficient, rationalized strategies for fitting the parameters (see Outstanding Questions). Although many such techniques exist for single-scale models (e.g., [32]), fitting multiscale models is a far less studied field and is often still performed manually.

Root Zonation Models and Their Connections

Auxin Dynamics

It has long been known that a longitudinal auxin gradient, with its maximum centered in the QC and SCN and tapering off along the meristem, plays a major role in root zonation dynamics. Following the **chemiosmotic theory of auxin transport**, auxin can only be actively exported from cells, resulting in an important role for exporters in auxin patterning. Therefore, to investigate the mechanistic basis of this root-tip auxin gradient Grieneisen *et al.* [23] developed a model incorporating the different root zones as well as experimental details on the pattern of polarly localized auxin-exporting PIN proteins. Specifically, the model includes a rootward orientation of PINs in the vasculature, apolar orientation of PINs in the columella, and shootward and laterally inward-oriented PINs in the outer tissue layers [23] (Figure 1, top row, second frame from left). The authors demonstrated that it is this particular layout, referred to as a **reverse fountain** or **reflux loop** in which laterally inward-oriented PINs cause recycling of upward-transported auxin back into the downward stream, that generates the auxin gradient. Furthermore, the model predicts that this effective recycling of auxin lends the root tip a degree of autonomy, enabling it to maintain its auxin maximum independently of auxin supply from the shoot for a substantial period of time. This prediction was subsequently experimentally validated using a **shoot cut** experiment, demonstrating at least 48 h maintenance of the auxin maximum.

A major question is how this PIN reflux arises *de novo* upon root-tip regeneration or the formation of a new lateral roots [33]. Interestingly, the PIN2 exporter essential for auxin reflux appears to be absent in the early stages of this process [34]. To investigate these issues, Mironova and coworkers [24,25] developed models which distinguish between PIN1, expressed in the middle vascular cell files with rootward polarity, PIN2, expressed in outer tissue files with shootward and inward polarity, and PIN3 that is expressed everywhere with an apolar pattern (Figure 1, top left). In addition, the models incorporate auxin-induced PIN expression and – at higher levels – auxin-induced PIN protein degradation (Figure 1, top left). This optimum curve-type auxin dependence was shown to allow vascular PIN1 to pattern an auxin maximum some distance from the root tip in a self-organized manner that enables repatterning after **root-tip excision** [24]. Put simply, if PIN1 expression extends all the way to

Reverse fountain: alternative name for the reflux loop that is based on the resemblance of the overall flow pattern to a horizontally mirrored fountain.
Root-tip excision: the root tip is cut off the root, thus removing the quiescent center and stem-cell niche, and leading to their subsequent regeneration.
Self-organization: pattern formation that arises from the internal dynamics of the system rather than from the imposition of external patterning controls.
Separated flow: similar to the reflux loop, there is PIN-mediated downward transport in the vasculature and upward transport in the outer tissues, but the inner and outer transport routes are separated because of the lateral outward rather than inward polarization of PINs in the cortex.
Shoot cut: the root is cut off from the shoot, depriving it of the nutrients and hormones it normally receives through the vasculature from the shoot, and which in the long term, but not within the first 48 h, results in termination of root growth and root death.

Key Figure

Synthesis of How Different Modeling Studies together Have Contributed to Our Understanding of Root Zonation Dynamics

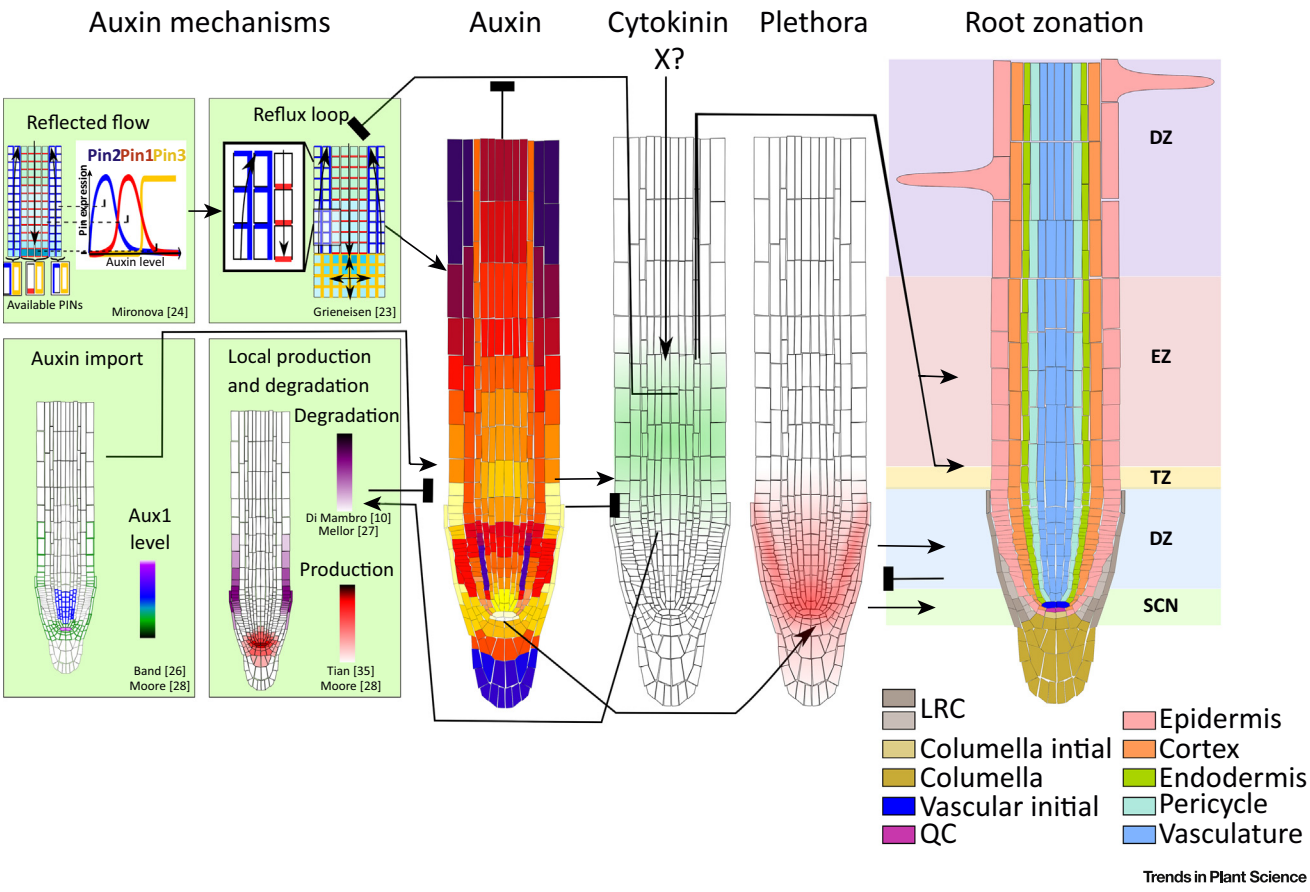

Figure 1. (Top left) The optimum auxin-dependence of PIN1 enables the self-organized patterning of a reflective flow, giving rise to a correctly positioned initial auxin maximum [22]. The different optimum auxin levels of PIN3 and PIN2 enable the subsequent bootstrapping of the full reflux loop [24], which transforms the localized auxin maximum into a spatially extended gradient [22]. (Bottom left) Tissue-specific Aux1 patterning [26,28], and localized production [34] and degradation [10,26], further shape the auxin pattern. (Middle, green) Whereas auxin induces both CK production and degradation [18–21], CK antagonizes auxin by repressing PINs (1, 3, and 7) [18] as well as by inducing localized auxin degradation [10], thereby patterning the auxin minimum demarcating the transition between meristem and elongation zone [10]. (Middle, red) Auxin signaling affects zonation through the activation of Plethora transcription factors which are required at high levels to maintain stemness and at intermediate levels for division potential [7]. (Left) Growth dynamics feed back on patterning gradients, predominantly the gradient of the slowly turning-over Plethoras, that together determine zonation boundaries [7]. See also [23,27,35]. Abbreviations: CK, cytokinin; DZ, differentiation zone; EZ, elongation zone; LRC, stem cell niche; QC, quiescent center; SCN, stem cell niche; TZ, transition zone.

the root tip, then auxin runs into a type of 'wall', causing high levels of auxin accumulation, and thereby inducing PIN1 degradation and retraction of the PIN1 domain. Next, once this so-called **reflected flow** mechanism has patterned PIN1, owing to their different optimal auxin values, PIN3 arises at the distal end of the PIN1 domain where auxin levels are highest, while PIN2 arises on the sides of the vasculature where PIN3 now delivers somewhat lower auxin levels. Together this establishes a reflux loop [25]. Because the model does not yet explain the spatial expression domains or polarity patterns of the different PIN types, it only partially answers how a

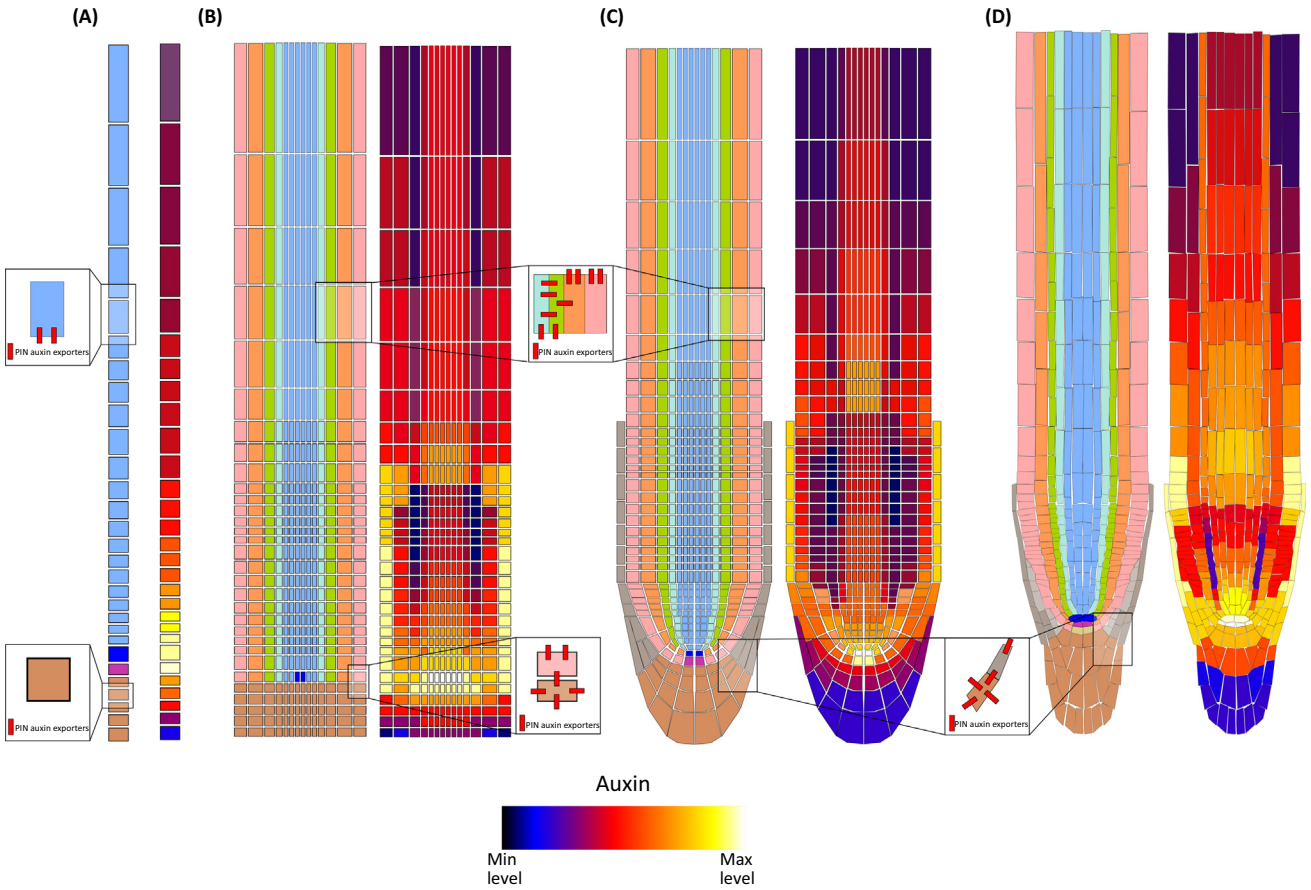

Figure 2. Tissue Layout of Root Models of Varying Levels of Complexity and Realism. As we move from left to right the models are able to capture more of the details of auxin transport, but simulating growth dynamics and other root-wide processes becomes more complex and computationally expensive. (A) 1D model: the root is represented as a single file of vascular cells. Downward-oriented flux of auxin between cells until the purple cell (left) generates a local auxin maximum near the tip of the cell file (right). (B) 2D rectangular model: the root is represented as a 2D cross-section containing all the distinct cell types of the root. As a simplification, cell files are assumed to run in parallel until they end either on the quiescent center (QC) or on the columella (left). A reflux type of PIN patterning (insets) creates an auxin maximum with an extended gradient (right). (C) Idealized wedge-shaped root models: the root is represented using a realistic wedge-shaped root tip in which the major cell files gradually curve inward towards the tip to converge on or next to the QC, and are locally protected by a columella and lateral root cap cells (left). Owing to the inward curving of cell files and the presence of a root cap, auxin reflux properties change subtly, resulting in a different auxin gradient pattern with an auxin maximum more focused around the QC, and the lateral root cap rather than meristematic epidermis cells having high auxin levels. (D) Image-based root morphology models: models that take an actual microscopy root picture as the input for the model root anatomy; these have a principle difference with idealized models that cell shapes are less regular and left–right asymmetries are present. Note that the auxin patterns predicted by the idealized and image-based models are highly similar.

reflux loop may arise in a self-organized manner. Even so, elegantly, the initial reflected flow mechanism explains the robustness of the auxin maximum against root-tip excision, while the resulting reflux loop explains robustness against shoot removal.

Although the reflux loop has been generally accepted as the mechanism underlying the root-tip auxin gradient, the lateral inward PIN positioning and its role in patterning the auxin gradient has been questioned [35]. Tian *et al.* [35] demonstrated that, assuming an outward-oriented cortical PIN pattern resulting in the **separated flow** instead of connecting the rootward and shootward auxin fluxes, PIN-mediated auxin transport alone would lead to an auxin maximum located below the QC. Feedback between auxin signaling, WOX5, and auxin production is necessary to

Box 1. Modeling Gene Expression and Auxin Dynamics

In Figure I we represent part of the hormonal crossregulation involved in root growth and development. Different models have incorporated different subsets of these hormones and interactions (see main text). To simulate the dynamical behavior of such a regulatory network, the models use ODEs that are suited to describe the continuous and gradual changes in the levels of 'network nodes' (variables), and how these depend on 'network edges' that describe the interactions among the different variables.

For instance, to model the dynamics of PIN1, we need to incorporate the effects of regulation on PIN1 (red edges in Figure I), namely the inhibition of PIN1 transcription by CK, the induction of PIN1 transcription by auxin, and the induction of PIN1 protein degradation by higher levels of auxin. Assuming that all regulatory effects depend in a saturating manner on the substance exerting the regulatory effect, this would result in the ODE of Equation I:

$$\frac{\mathrm{dPin1}}{\mathrm{dt}} = \mathrm{p}_b + \mathrm{p}_{\mathrm{reg}} * \left(1 - \left(\frac{\mathrm{Cytokinin}^2}{\mathrm{Cytokinin}^2 + \mathrm{K}_{\mathrm{cyt}}^2} * \left(1 - \frac{\mathrm{K}_{\mathrm{aux}}^2}{\mathrm{Auxin}^2 + \mathrm{K}_{\mathrm{aux}}^2}\right)\right)\right) - \left(d_b + \frac{d_{\mathrm{auxin}} * \mathrm{Auxin}^2}{\mathrm{Auxin}^2 + \mathrm{K}_{degr}^2}\right) * \mathrm{Pin1} \quad [\mathrm{I}]$$

where p_b is the baseline PIN1 transcriptional level, p_{reg} is the maximum induced transcriptional level, K_{cyt} is the CK concentration at which repression of transcription is half-maximal, K_{aux} is the auxin level at which the relief from CK repression by auxin is half-maximal, d_b is the baseline PIN1 protein degradation rate, d_{auxin} is the auxin-induced additional degradation rate, and K_{degr} is the auxin concentration level at which auxin-induced degradation is half-maximal. Furthermore, because intermediate auxin levels increase and higher auxin levels reduce overall PIN1 protein levels, it should hold that $K_{aux} < K_{degr}$.

Importantly, in the above case a single ODE is used to describe PIN1 dynamics without distinguishing between the production of PIN1 mRNA and its subsequent translation into PIN1 protein. Such an approach is often taken to reduce the number of variables in a model, increasing both the computational efficiency of the model as well as reducing the number of parameters for which values need to be determined. Nonetheless, in other cases it may be relevant to explicitly model both PIN1 mRNA and protein, for example if transcription and translation have very different timescales, which would result in Equations II and III:

$$\frac{\mathrm{dPin1}_{\mathrm{mRNA}}}{\mathrm{dt}} = \mathrm{p}_b + \mathrm{p}_{\mathrm{reg}} * \left(1 - \left(\frac{\mathrm{Cytokinin}^2}{\mathrm{Cytokinin}^2 + \mathrm{K}_{\mathrm{cyt}}^2} * \left(\frac{\mathrm{K}_{\mathrm{aux}}^2}{\mathrm{Auxin}^2 + \mathrm{K}_{\mathrm{aux}}^2}\right)\right)\right) - d_{\mathrm{mRNA}} * \mathrm{dPin1}_{\mathrm{mRNA}} \quad [\mathrm{II}]$$

$$\frac{\mathrm{dPin1}_{\mathrm{prot}}}{\mathrm{dt}} = \mathrm{p}_{\mathrm{mRNA}} * \mathrm{Pin1}_{\mathrm{mRNA}} - \left(d_b + \frac{d_{\mathrm{auxin}} * \mathrm{Auxin}^2}{\mathrm{Auxin}^2 + \mathrm{K}_{\mathrm{degr}}^2}\right) * \mathrm{Pin1}_{\mathrm{prot}} \quad [\mathrm{III}]$$

where d_{mRNA} is the degradation rate of PIN1 mRNA and p_{mRNA} is the translation rate of mRNA into protein.

Many of the factors relevant for root development, hormones, but also peptides and miRNAs, are transported in between cells. Thus, to describe the dynamics of these signaling molecules, transport processes have to be incorporated in the ODEs. Let us illustrate this for auxin, for which transport processes are often incorporated into root models. Equation IV captures the regulatory effects impacting on auxin, involving either transport or metabolism (green edges in Figure I).

$$\frac{\mathrm{dAuxin}_{\mathrm{ij}}}{\mathrm{dt}} = \mathrm{p}_b + \frac{p_{\mathrm{wox5}} * \mathrm{WOX5}^2}{\mathrm{WOX5}^2 + \mathrm{K}_{\mathrm{WOX5}}^2} - \left(d_b + \frac{d_{\mathrm{GH317}} * \mathrm{GH317}^2}{\mathrm{GH317}^2 + \mathrm{K}_{\mathrm{GH317}}^2}\right) * \mathrm{Auxin}_{\mathrm{ij}} + \sum_{i',j'} (i_{\mathrm{pas}} + \mathrm{i}_{\mathrm{act}} * \mathrm{Aux1}) * \mathrm{Auxin}_{i'j'} - \sum_{i',j'} (\mathrm{e}_{\mathrm{bas}} + \mathrm{e}_{\mathrm{act}} * \mathrm{Pin}) \mathrm{Auxin}_{\mathrm{ij}} \quad [\mathrm{IV}]$$

where p_b and d_b denote basal production and degradation respectively, p_{WOX5} and d_{GH317} stand for the maximum additional production and degradation under WOX5 and GH317 regulation, with K_{WOX5} and K_{GH317} being the respective saturation constants. In addition, i_{pas} and i_{act} describe the rate of passive transmembrane auxin uptake, and active AUX1-mediated auxin uptake, while e_{bas} describes the baseline non-PIN-mediated active export of auxin that is assumed to be constant and similar for all cells, and e_{act} describes the active PIN-mediated auxin export.

Although in most models auxin dynamics are simulated using the above type of ODE, in some models a subcellular resolution is applied, incorporating also auxin diffusion within cells or cell walls. In these models, so-called **partial differential equations** (PDEs) are applied, as exemplified in Equation V:

$$\frac{\partial \mathrm{Auxin}_{\mathrm{ij}}}{\partial \mathrm{t}} = \mathrm{p}_b + \frac{p_{\mathrm{wox5}} * \mathrm{WOX5}^2}{\mathrm{WOX5}^2 + \mathrm{K}_{\mathrm{WOX5}}^2} - \left(d_b + \frac{d_{\mathrm{Gh}} * \mathrm{GH317}^2}{\mathrm{GH317}^2 + \mathrm{K}_{\mathrm{GH317}}^2}\right) * \mathrm{Auxin}_{\mathrm{ij}} + \sum_{i',j'} (i_{\mathrm{pas}} + \mathrm{i}_{\mathrm{act}} * \mathrm{Aux1}) * \mathrm{Auxin}_{i'j'} - \sum_{i',j'} (\mathrm{e}_{\mathrm{bas}} + \mathrm{e}_{\mathrm{act}} * \mathrm{Pin}) * \mathrm{Auxin}_{\mathrm{ij}} + D\left(\frac{\partial \mathrm{Auxin}}{\partial x^2} + \frac{\partial^2 \mathrm{Auxin}}{\partial y^2}\right) \quad [\mathrm{V}]$$

where D determines the diffusion speed of auxin in the *x* and *y* directions, and diffusion only occurs between grid points that are either all inside the wall or all inside the cellular cytoplasm.

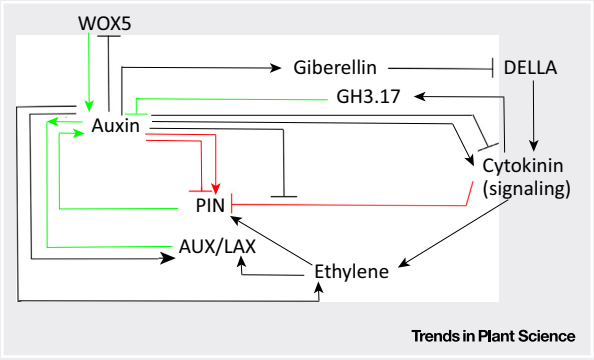

Figure I. Simplified Depiction of the Hormone Crossregulatory Network Involved in Root Developmental Patterning. Positive interactions are indicated by arrows, and negative interactions by perpendicular bars. Red edges indicate regulatory effects on PIN; green edges indicate regulatory effects on auxin.

position the maximum at the QC (Figure 1, bottom, second frame from the left). Intriguingly, experimental data suggest that cortical cells have both inward- and outward-oriented lateral PINs, implying that both reflux and locally regulated auxin production are relevant [36,37].

The models discussed so far have focused on the impact of auxin exporters on root-tip auxin patterning, assuming uniform auxin import into cells. However, although auxin can passively enter cells, AUX/LAX importers substantially enhance auxin uptake and have tissue-specific distribution patterns. The relevance of these importers was studied in an elegant modeling study that incorporated realistic root-tip anatomy and cell type-specific PIN and AUX/LAX patterns [26] (Figure 1, bottom left). The authors demonstrated that the presence of AUX/LAX importers in the lateral root cap and elongating epidermal cells significantly impacted on auxin patterning, improving the agreement between simulated and experimental patterns.

In addition, the above models assumed constant, homogeneous auxin production and degradation. In another study, Band and coworkers investigated the relative contributions of different auxin degradation routes (oxidation versus conjugation) and their regulation to root-tip auxin distribution [27]. The study revealed that, although oxidation is slowly induced at low auxin levels, enabling for example environmentally induced auxin changes to elicit a response before being removed, the conjugation route is rapidly induced at higher auxin levels, ensuring quick recovery in cases of substantial auxin increases. Intriguingly, the study predicted that, in the case of a defective oxidation route, not only does conjugation increase, as would be expected, but auxin production also significantly increases. This suggests an additional, negative feedback of oxidation but not conjugation products on auxin production that under wild-type conditions would contribute to homeostasis, but in these mutants causes an overproduction of auxin. This differential feedback of oxidation and conjugation products has thus far not been experimentally tested.

The importance of auxin importers and metabolism was further supported by work from the group of Lindsey [29] (Figure 1, bottom, two leftmost frames). These authors showed that a simple, homogeneous change in auxin-exporter levels would require a highly complex, non-homogeneous change in auxin-importer levels to retrieve the same auxin pattern, thus elegantly

demonstrating the need for coordinated regulation of auxin importers and exporters. In addition, the model correctly predicted regions of high auxin biosynthesis around the QC and in the columella that are necessary to produce correct auxin patterns. Interestingly, the production of the auxin precursor indole-3-butyric acid (IBA) in the lateral root cap was not recovered, possibly owing to high auxin degradation in this area [10].

The Interplay of Auxin with Other Hormones

Many hormones in addition to auxin impact on root zonation dynamics. Among these, the auxin antagonist CK is of crucial importance. The tug of war between auxin and CK determines the transition from division zone to elongation and differentiation zone. Thus, a crucial question concerns how auxin and CK signaling domains are patterned, affect one another, and are modulated by other hormones.

Modeling work by the group of Lindsey has focused on the hormonal crosstalk between auxin, CK, and ethylene, and the role of such crosstalk role in root development. Initially the group developed single-cell models [38], subsequently extending their work to a spatial root model [28]. The model incorporates hormone transport, crossregulation of hormone biosynthesis and signaling, hormonal regulation of AUX1 and PINs, as well as inhibition of PIN internalization by auxin, resulting in a highly complex crossregulatory network. Using this model, patterning of auxin, ethylene, and PIN1 could be correctly reproduced, but CK patterning could not be correctly simulated. Although this could indicate a lack of necessary data, it is also important to note that the model does not distinguish CK hormone levels from downstream CK signaling. It is well known that the distal root tip is a major zone of CK production [39,40], but for example the ARRs (Arabidopsis Response Regulators) necessary for CK signaling are predominantly expressed in the elongation zone [11]. Thus, to understand the antagonistic role of CK, investigation of CK transport as well as the patterning of CK downstream signaling components is likely to be essential.

Modeling of auxin–CK interactions in the developing root tip was started by the groups of King and Bennett, again building up from an initial single-cell model [41] to a 1D cell-file model [22] and subsequently to 2D root models [31]. The root model focuses on the interactions between auxin, GA, and CK. CK signaling is simulated in detail, incorporating auxin-dependent CK biosynthesis, AHK receptor phosphorylation, and activation of ARRs. Downstream of ARR signaling is SHY2 which suppresses the auxin-transporting PIN proteins (Figure 1, middle), while antagonism in the reverse direction arises from auxin repression of SHY2 as well as of CK biosynthesis. Although ARR12 is assumed to be active by default, ARR1 induction requires DELLAs. The model nicely demonstrates how, as development proceeds and GA levels drop, derepressed DELLAs activate ARR1 which subsequently puts a halt to meristem expansion [12,42]. However, without any further interactions, ARR1 activation would also occur inside the meristem, causing meristem collapse. The model thus predicts the existence of an additional factor that is necessary to restrict ARR expression to the elongation zone (Figure 1, middle, and top, second from left). This factor has not yet been identified.

In the most recent model focusing on auxin–CK antagonism, instead of incorporating all details of CK production and signal transduction, only the essential downstream CK signaling is incorporated. The model predicts a CK signaling-induced auxin minimum that separates the QC-focused meristematic auxin gradient from the secondary rise in auxin that signals the start of the elongation zone. The occurrence of this auxin minimum was subsequently experimentally confirmed. Thus, although one might naively expect that CK – an auxin antagonist – simply provides a shootward boundary for the auxin gradient, it has a much

stronger impact on shaping the auxin gradient. A major difference between this and earlier models is the incorporation of CK-induced expression of the auxin-degrading GH3.17 enzyme, based on experimental data (Figure 1, middle, and bottom, second from left). In addition, CK was simulated to repress not only vascular but overall PIN levels, again based on data. Together, these differences may explain why earlier studies did not uncover this patterning of the auxin minimum. In agreement with the earlier discussed models, the model needed to superimpose the region where CK signaling is active, thus leaving open the question of how this domain is patterned.

Adding Growth

To date, most published models focus on explaining patterns of hormone activity, taking these patterns as a proxy for the sizes of the developmental zones and hence for root growth rates. Importantly, these hormone gradients differ fundamentally from the positional morphogen gradients that drive the differentiation of a field of cells, as first proposed by Lewis Wolpert [43]. First, in the presence of growth, stable spatial domains must be patterned despite individual cells sequentially traversing these zones. Second, growth dilutes and displaces signaling molecules, thereby feeding back on the patterning processes (Box 2).

The first model investigating the impact of growth simulated a 1D file of elongation zone cells, incorporating expansion-driven dilution of GA and its effects on downstream signaling via GID and DELLA [44]. The model shows that, given limited GA mobility between cells, diffusion cannot smooth out the dilution-driven decline. This is in stark contrast to auxin patterning in which active PIN- and AUX/LAX-mediated transport between cells overrides any possible dilution effects. More recent results indicate that GA levels in fact increase with cell size [45] and strongly depend on cellular GA uptake [46]. Although this indicates that growth-induced dilution does not dominate overall GA dynamics, the model remains a powerful illustration of how growth dynamics may impact on patterning.

Paradoxically, the root-tip auxin gradient that guides stable root zonation also needs to swiftly become asymmetric to enable root tropisms [47]. Intriguingly, the auxin-inducible PLETHORA

Box 2. The Impact of Growth on Patterning Processes

According to the classical morphogen gradient model first proposed by Lewis Wolpert [43], the positional information encoded in different morphogen levels at different positions across a static tissue enables cells (or nuclei) to obtain different and temporally stable cell fates. At a mechanistic level, cells perceive their local morphogen concentration, and translate this into a concentration-dependent gene expression program that determines the type of cell they become (Figure IA). By contrast, in root developmental zonation the hormone gradients dictate the behavior of cells by controlling where in the root cells undergo slow stem-cell divisions or rapid transit amplifying divisions, elongation, and differentiation. Furthermore, because of cell growth and division, new cells are continuously generated, thereby displacing older cells shootward. Thus, although the different developmental zones need to be stably patterned, individual cells traverse through these zones as they age instead of residing at a specific, fixed location (see red cell followed over time in Figure IB). Importantly, generating stable spatial domains in a growing tissue may thus pose additional constraints on the gradient patterning mechanisms.

Furthermore, growth dynamics may actively feed back on the hormone and protein gradients that controlling them. First, at the individual cell level, cell growth and expansion can, owing to volume increase, lead to a dilution of hormone and protein concentrations (Figure IC). The severity of this dilution is dependent on the relative rate of volume increase compared to the turnover rate of the protein (Figure ID). Second, owing to the growth of the tissue as a whole, individual cells are displaced shootward, allowing those signaling molecules that have a low turnover rate to travel outside their local domain of production (Figure IC). The effects of both dilution and displacement processes are illustrated in Figure IB for a situation where signaling molecule production only occurs inside the QC and stem cells neighboring the QC, and the signaling molecule has a low turnover rate.

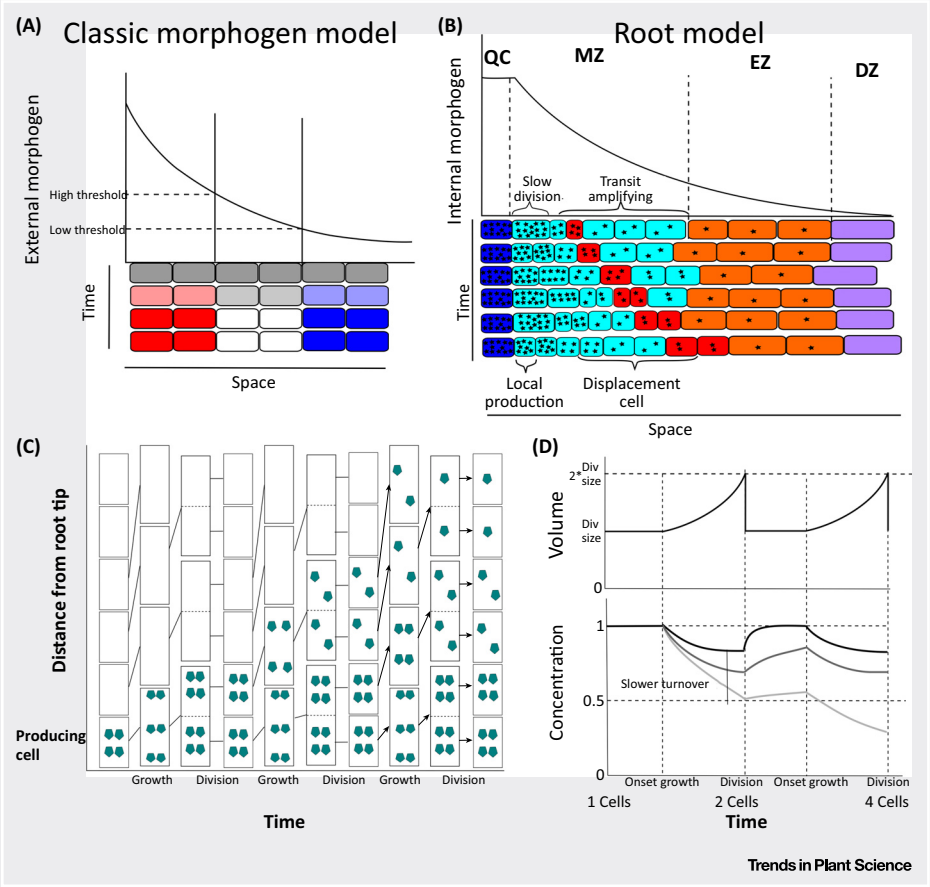

Figure I. Growth and Patterning Dynamics. (A) Patterning of a static tissue by a morphogen gradient. (B) Patterning of developmental zones in the growing tissue of a root tip. (C) Illustration of the effect of growth on diluting signaling molecule concentrations through volume increase (e.g., left 2 columns), and the effect on displacing signaling molecules owing to the growth of the underlying cells. (D) (Top) Changes in cell volume produced by growth and subsequent division. (Bottom) Corresponding changes in signaling molecule concentrations as a result of the above-shown changes in cell volume for different signaling molecule turnover rates. Abbreviations: DZ, differentiation zone; EZ, elongation zone; MZ, meristematic zone; QC, quiescent center.

(PLT) transcription factors show a graded distribution highly reminiscent of the auxin gradient, and appear to control root zonation in conjunction with auxin (Figure 1, second panel from right). To investigate how the interplay between auxins and PLTs may solve the auxin control paradox, additional data were first required. Experimentally it was shown that, although auxins control the rates of division, expansion, and differentiation, PLT levels dictate where cells divide, expand, or differentiate [7]. In addition, only prolonged exposure to high auxin levels was sufficient to induce PLT transcription, while the resulting PLT proteins were demonstrated to move through plasmodesmata to neighboring cells. These findings were built into a root model that explicitly incorporated cell, division, elongation, and differentiation, as well as the dilution and displacement effects these have [7]. The model predicted that the PLT transcriptional domain is restricted to the region around the QC and has no role in gradient formation. Instead, the model predicted that a highly stable PLT protein would enable formation of a PLT protein gradient through cell-to-cell movement and growth-driven cell displacement (Box 2). Both predictions were experimentally confirmed. The model next predicted that, because of the slow

timescales involved in PLT gradient patterning, tropism-induced auxin asymmetry does not lead to PLT asymmetry. Again, these findings were confirmed experimentally. This resolves the earlier-mentioned paradox: because of the division of labor in which auxin directly affects growth, division, and elongation rates, but only indirectly via the slow PLTs, affects zonation boundaries, tropisms do not perturb developmental zonation. Finally, it is known that, beyond a given auxin level, division rates decrease and root meristems also shrink. With the model developed, this can be explained from the fact that reduced divisions cause shortening of the division-dependent PLT gradient that controls meristem size. The model thus illustrates how incorporating the dependence of rates of cellular processes on hormones gives rise to important additional feedback.

Concluding Remarks and Future Perspectives

Over the past decade computational models have contributed significantly to our understanding of the processes governing root growth and development. It is now generally accepted that a reverse-fountain-type pattern of the auxin-exporting, polar PIN proteins plays a dominant role in generating the root-tip auxin gradient [23]. In addition, important roles for auxin-importing AUX/LAX proteins [26,29] and for localized, regulated of auxin production and degradation have been demonstrated [10,27,29]. Models could provide these insights by enabling us to investigate the consequences of, for example, differently polarized PIN proteins or differently expressed auxin-degrading enzymes, perturbations that are not always easy to achieve experimentally. Modeling has also taught us that CK does not merely antagonize auxin, thereby delimiting the auxin gradient, but divides the auxin pattern into a QC-centered strong gradient and a more modest auxin increase in the elongation zone [10]. At the same time, modeling studies have highlighted how a gap in our current knowledge prevents us from mechanistically simulating the spatial domain of CK signaling [31] (see Outstanding Questions). Finally, a few models have started to incorporate the additional complexities of growth, and its effects on diluting and displacing signaling molecules [7,44]. As a case in point, the slow, division- and cell-to-cell movement-dependent gradient of the PLT proteins was shown to enable auxins to control both stable developmental zonation and fast tropisms.

An interesting extension for current models that will be in reach in the near future is to incorporate further details of auxin transport. Recent studies demonstrate that non-phosphorylated PINs have limited auxin transport capacity, making PIN phosphorylation an important regulatory mechanism for auxin transport [48,49]. In addition, once phosphorylated, PINs were shown to differ significantly in their maximum rates of auxin transport [48,49]. Similar data for AUX/LAX auxin importers can be expected. In addition, although current models do not explicitly model ABCB/PGP auxin transporters, these not only complement PIN-mediated auxin transport [50] but also influence the stability of PINs at the plasma membrane [51]. Incorporating this knowledge may enable further closing of the remaining gap between simulated and experimentally observed auxin patterns. Similarly, it is now also clear for other hormones that active transporters exist that have a major impact on patterning the domain of hormone action, and that hence need to be taken in to account in our models. As an example, the predominant localization of GA in the endodermis of the elongation zone [45] was recently shown to depend on localized active transport [52] via a SWEET sugar transporter capable of importing GA [46].

In addition to these relatively straightforward extensions, many more fundamental questions remain to be addressed. Computational modeling has shown that, to maintain non-disruptive symplastic growth, only limited and short-lived differences in longitudinal elongation are tolerated [53]. Nonetheless, different hormones are accumulated, perceived, and exert their

Outstanding Questions

Biological

Although computational studies show the importance of CKs for delimiting and shaping the auxin gradient, it is currently unclear how the domain of CK signaling is itself regulated, particularly given that CK production and signaling appear to occur in spatially largely distinct domains.

During root development, upon germination the root meristem first needs to be activated, next it needs to grow out, and finally it needs to stop growing and maintain a stable size. Exactly how auxin–CK interactions allow this initial bootstrapping and subsequent taming of a new meristem is still poorly understood.

Patterning is fundamentally different in static versus growing tissues, with growth giving rise to dilution and displacement of signaling molecules. In addition, although the spatial domains need to be stably maintained in growing tissues, the individual cells flux through these domains as they age. It is currently incompletely understood how growth dynamics precisely affects the requirements and constraints imposed on patterning mechanisms.

Computational

Different computational research groups have applied different spatial resolutions (subcellular versus cellular) when simulating auxin-transport dynamics. A comparative study will be necessary to investigate the effects these differences have on precise modeling outcomes, and under what conditions each type of approach should be preferred.

Multiscale models simulating the crossregulatory hormonal networks that pattern root development contain a large number of parameters for which little data are often available. Development of fitting algorithms tailored specifically for multilevel models would enable more efficient and rationalized fitting of these models.

control on root growth in distinct tissues, with for example auxin, ethylene, and brassinosteroids impinging on the epidermis [14,53], ABA acting on the cortex [54], and GA affecting the endodermis [45]. This raises the question of how root growth is coordinated in the radial direction. Tissue mechanics are likely to play in important role in this coordination [55], with hormone-induced growth changes in one tissue being both transmitted to and dampened by surrounding tissues.

On a very different note, root growth does not depend only on hormonal and transcription factor patterning of the root into developmental zones, but is also strongly dependent on the energy available for root growth. Thus far, investigations on plant development and plant physiology, in both experimental and modeling studies, have remained largely separated. Interestingly, delivery of sugar to the root tip induces auxin synthesis [56]. Furthermore, sugars influence auxin transport and patterning [57]. In addition to glucose enhancement of auxin-dependent cell division and growth, sucrose itself also impinges on these processes [58]. Together this gives rise to a coherent feedforward type of regulatory architecture that coordinates the developmental and energy-guided control of root growth. Most likely, many more levels of integration between these two control routes remain to be discovered. Developing models that bridge the divide between development and physiology is thus likely to be a fruitful direction for new research.

However, we would like to argue that, first and foremost, models aimed at explaining the dynamic, self-organized nature and developmental timeline of root development are needed (see Outstanding Questions). Because current models have not yet answered how CK signaling is patterned [10,31], we currently cannot fully explain how auxin and CK domains self-organize during embryogenesis and *de novo* organ formation, or change in response to environmental conditions. New modeling studies should be aimed at answering these questions. Incorporation of growth processes will be an essential prerequisite for these models because the initial patterning of a new meristem will lead to meristem activation, with the resulting growth fueling displacement and dilution processes that feed back on the patterning process. Furthermore, although patterning of spatial domains in a non-growing tissue entails that cells obtain a position-specific, stable fate, individual cells in growing tissues need to change their behavior as they traverse these zones. Explicitly incorporating these dilution, displacement, and cell-behavior changes into our models will undoubtedly reveal additional constraints and requirements for such dynamic patterning processes. These insights will be crucial for unraveling their underlying logic.

Acknowledgments

This work was supported by The Netherlands Scientific Organization (Nederlandse Organisatie voor Wetenschappelijk Onderzoek) through grant 864.14.003 to J.P.R. and K.t.T.

References

1. Goh, T. *et al.* (2014) Systems biology approaches to understand the role of auxin in root growth and development. *Physiol. Plant.* 151, 73–82
2. van den Berg, T. *et al.* (2016) Modeling halotropism: a key role for root tip architecture and reflux loop remodeling in redistributing auxin. *Development* 143, 3350–3362
3. Nakamasu, A. *et al.* (2009) Interactions between zebrafish pigment cells responsible for the generation of Turing patterns. *Proc. Natl. Acad. Sci. U. S. A.* 106, 8429–8434
4. Fisher, A.P. and Sozzani, R. (2016) Uncovering the networks involved in stem cell maintenance and asymmetric cell division in the *Arabidopsis* root. *Curr. Opin. Plant Biol.* 29, 38–43
5. Ubeda-Tomas, S. and Bennett, M.J. (2010) Plant development: size matters, and it's all down to hormones. *Curr. Biol.* 20, R511–R513
6. Ivanov, V.B. and Dubrovsky, J.G. (2013) Longitudinal zonation pattern in plant roots: conflicts and solutions. *Trends Plant Sci.* 18, 237–243
7. Mähönen, A.P. *et al.* (2014) PLETHORA gradient formation mechanism separates auxin responses. *Nature* 515, 125–129
8. Sabatini, S. *et al.* (1999) An auxin-dependent distal organizer of pattern and polarity in the *Arabidopsis* root. *Cell* 99, 463–472
9. Blilou, I. *et al.* (2005) The PIN auxin efflux facilitator network controls growth and patterning in *Arabidopsis* roots. *Nature* 433, 39–44

10. Di Mambro, R. *et al.* (2017) Auxin minimum triggers the developmental switch from cell division to cell differentiation in the *Arabidopsis* root. *Proc. Natl. Acad. Sci. U. S. A.* 114, E7641–E7649
11. Dello Ioio, R. *et al.* (2007) Cytokinins determine *Arabidopsis* root-meristem size by controlling cell differentiation. *Curr. Biol.* 17, 678–682
12. Moubayidin, L. *et al.* (2010) The rate of cell differentiation controls the *Arabidopsis* root meristem growth phase. *Curr. Biol.* 20, 1138–1143
13. Moubayidin, L. *et al.* (2013) Spatial coordination between stem cell activity and cell differentiation in the root meristem. *Dev. Cell* 26, 405–415
14. Vaseva, I.I. *et al.* (2018) The plant hormone ethylene restricts *Arabidopsis* growth via the epidermis. *Proc. Natl. Acad. Sci. U. S. A.* 115, E4130–E4139
15. Ubeda-Tomás, S. *et al.* (2009) Gibberellin signaling in the endodermis controls *Arabidopsis* root meristem size. *Curr. Biol.* 19, 1194–1199
16. Thole, J.M. *et al.* (2014) Abscisic acid regulates root elongation through the activities of auxin and ethylene in *Arabidopsis thaliana*. *G3 (Bethesda)* 4, 1259–1274
17. Hacham, Y. *et al.* (2011) Brassinosteroid perception in the epidermis controls root meristem size. *Development* 138, 839–848
18. Miyawaki, K. *et al.* (2004) Expression of cytokinin biosynthetic isopentenyltransferase genes in *Arabidopsis*: tissue specificity and regulation by auxin, cytokinin, and nitrate. *Plant J.* 37, 128–138
19. Dello Ioio, R. *et al.* (2008) A genetic framework for the control of cell division and differentiation in the root meristem. *Science* 322, 1380–1384
20. Nordström, A. *et al.* (2004) Auxin regulation of cytokinin biosynthesis in *Arabidopsis thaliana*: a factor of potential importance for auxin–cytokinin-regulated development. *Proc. Natl. Acad. Sci. U. S. A.* 101, 8039–8044
21. Affonso, V.R. *et al.* (2009) Influence of growth regulators in biomass production and volatile profile of *in vitro* plantlets of *Thymus vulgaris* L. *J. Agric. Food Chem.* 57, 6392–6395
22. Muraro, D. *et al.* (2013) The role of auxin and cytokinin signalling in specifying the root architecture of *Arabidopsis thaliana*. *J. Theor. Biol.* 317, 71–86
23. Grieneisen, V.A. *et al.* (2007) Auxin transport is sufficient to generate a maximum and gradient guiding root growth. *Nature* 449, 1008–1013
24. Mironova, V.V. *et al.* (2010) A plausible mechanism for auxin patterning along the developing root. *BMC Syst. Biol.* 4, 98
25. Mironova, V.V. *et al.* (2012) Combined *in silico/in vivo* analysis of mechanisms providing for root apical meristem self-organization and maintenance. *Ann. Bot.* 110, 349–360
26. Band, L.R. *et al.* (2014) Systems analysis of auxin transport in the *Arabidopsis* root apex. *Plant Cell* 26, 862–875
27. Mellor, N. *et al.* (2016) Dynamic regulation of auxin oxidase and conjugating enzymes AtDAO1 and GH3 modulates auxin homeostasis. *Proc. Natl. Acad. Sci. U. S. A.* 113, 11022–11027
28. Moore, S. *et al.* (2015) Spatiotemporal modelling of hormonal crosstalk explains the level and patterning of hormones and gene expression in *Arabidopsis thaliana* wild-type and mutant roots. *New Phytol.* 207, 1110–1122
29. Moore, S. *et al.* (2017) A recovery principle provides insight into auxin pattern control in the *Arabidopsis* root. *Sci. Rep.* 7, 43004
30. Cruz-Ramírez, A. *et al.* (2012) A bistable circuit involving SCARECROW–RETINOBLASTOMA integrates cues to inform asymmetric stem cell division. *Cell* 150, 1002–1015
31. Muraro, D. *et al.* (2016) A multi-scale model of the interplay between cell signalling and hormone transport in specifying the root meristem of *Arabidopsis thaliana*. *J. Theor. Biol.* 404, 182–205
32. Vernon, I. *et al.* (2018) Bayesian uncertainty analysis for complex systems biology models: emulation, global parameter searches and evaluation of gene functions. *BMC Syst. Biol.* 12, 1
33. Sauer, M. *et al.* (2006) Canalization of auxin flow by Aux/IAA–ARF-dependent feedback regulation of PIN polarity. *Genes Dev.* 20, 2902–2911
34. Benková, E. *et al.* (2003) Local, efflux-dependent auxin gradients as a common module for plant organ formation. *Cell* 115, 591–602
35. Tian, H. *et al.* (2014) Shaping a root system: regulating lateral versus primary root growth. *Trends Plant Sci.* 19, 426–431
36. Kim, J.-Y. *et al.* (2010) Identification of an ABCB/P-glycoprotein-specific inhibitor of auxin transport by chemical genomics. *J. Biol. Chem.* 285, 23309–23317
37. Jiang, K. *et al.* (2016) Salt stress affects the redox status of *Arabidopsis* root meristems. *Front. Plant Sci.* 7, 81
38. Liu, J. *et al.* (2010) Modelling and experimental analysis of hormonal crosstalk in *Arabidopsis*. *Mol. Syst. Biol.* 6, 373
39. Singh, S. *et al.* (2017) Sirtinol, a Sir2 protein inhibitor, affects stem cell maintenance and root development in *Arabidopsis thaliana* by modulating auxin–cytokinin signaling components. *Sci. Rep.* 7, 42450
40. Casson, S.A. *et al.* (2002) The POLARIS gene of *Arabidopsis* encodes a predicted peptide required for correct root growth and leaf vascular patterning. *Plant Cell* 14, 1705–1721
41. Muraro *et al.* (2011) The influence of cytokinin–auxin cross-regulation on cell-fate determination in *Arabidopsis thaliana* root development. *J. Theor. Biol.* 283, 152–167
42. Marín-de la Rosa, N. *et al.* (2015) Genome wide binding site analysis reveals transcriptional coactivation of cytokinin-responsive genes by DELLA proteins. *PLoS Genet.* 11, e1005337
43. Wolpert, L. (1969) Positional information and the spatial pattern of cellular differentiation. *J. Theor. Biol.* 25, 1–47
44. Band, L.R. *et al.* (2012) Growth-induced hormone dilution can explain the dynamics of plant root cell elongation. *Proc. Natl. Acad. Sci. U. S. A.* 109, 7577–7582
45. Rizza, A. *et al.* (2017) In vivo gibberellin gradients visualized in rapidly elongating tissues. *Nat. Plants* 3, 803–813
46. Binenbaum, J. *et al.* (2018) Gibberellin localization and transport in plants. *Trends Plant Sci.* 23, 410–421
47. Band, L.R. *et al.* (2012) Multiscale systems analysis of root growth and development: modeling beyond the network and cellular scales. *Plant Cell* 24, 3892–3906
48. Zourelidou, M. *et al.* (2014) Auxin efflux by PIN-FORMED proteins is activated by two different protein kinases, D6 PROTEIN KINASE and PINOID. *eLife* 3, e02860
49. Marhava, P. *et al.* (2018) A molecular rheostat adjusts auxin flux to promote root protophloem differentiation. *Nature* 558, 297–300
50. Cho, M. and Cho, H.-T. (2013) The function of ABCB transporters in auxin transport. *Plant Signal. Behav.* 8, e22990
51. Titapiwatanakun, B. *et al.* (2009) ABCB19/PGP19 stabilises PIN1 in membrane microdomains in *Arabidopsis*. *Plant J.* 57, 27–44
52. Shani, E. *et al.* (2013) Gibberellins accumulate in the elongating endodermal cells of *Arabidopsis* root. *Proc. Natl. Acad. Sci. U. S. A.* 110, 4834–4839
53. De Vos, D. *et al.* (2014) Putting theory to the test: which regulatory mechanisms can drive realistic growth of a root? *PLoS Comput. Biol.* 10, e1003910
54. Dietrich, D. *et al.* (2017) Root hydrotropism is controlled via a cortex-specific growth mechanism. *Nat Plants* 3, 17057
55. Dyson, R.J. *et al.* (2014) Mechanical modelling quantifies the functional importance of outer tissue layers during root elongation and bending. *New Phytol.* 202, 1212–1222
56. Sairanen, I. *et al.* (2012) Soluble carbohydrates regulate auxin biosynthesis via PIF proteins in *Arabidopsis*. *Plant Cell* 24, 4907–4916
57. Raya-González, J. *et al.* (2017) The MEDIATOR genes MED12 and MED13 control *Arabidopsis* root system configuration influencing sugar and auxin responses. *Plant Mol. Biol.* 95, 141–156
58. Xiong, Y. *et al.* (2013) Glucose–TOR signalling reprograms the transcriptome and activates meristems. *Nature* 496, 181–186