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The Systems Biology of Lateral Root Formation: Connecting the Dots

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ABSTRACT

The root system is a major determinant of a plant's access to water and nutrients. The architecture of the root system to a large extent depends on the repeated formation of new lateral roots. In this review, we discuss lateral root development from a systems biology perspective. We focus on studies combining experiments with computational modeling that have advanced our understanding of how the auxin-centered regulatory modules involved in different stages of lateral root development exert their specific functions. Moreover, we discuss how these regulatory networks may enable robust transitions from one developmental stage to the next, a subject that thus far has received limited attention. In addition, we analyze how environmental factors impinge on these modules, and the different manners in which these environmental signals are being integrated to enable coordinated developmental decision making. Finally, we provide some suggestions for extending current models of lateral root development to incorporate multiple processes and stages. Only through more comprehensive models we can fully elucidate the cooperative effects of multiple processes on later root formation, and how one stage drives the transition to the next.

Key words: *Arabidopsis*, lateral root, developmental stages, auxin signaling modules, systems biology

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INTRODUCTION

The root system of plants determines a plant's access to water and nutrients, as well as its anchorage to the soil substratum. While taking up resources, roots are involved in subterranean competition with other plants, complex collaborations with beneficial bacteria and fungi, as well as arms races with detrimental microbes (Lundberg et al., 2012; Rey and Schornack, 2013; Evangelisti et al., 2014; Hortal et al., 2017; Durán et al., 2018). The overall architecture of the root system and the ability to adapt this architecture in response to environmental conditions, called plasticity, is a major determinant of overall plant fitness (Den Herder et al., 2010; Eshel and Kafkafi, 2013; Tian et al., 2014; Rogers and Benfey, 2015). It has been suggested that for a second green revolution, required to feed more people in a more sustainable manner, understanding how plant root system architecture enables plants to adapt to their environment, and how we may enhance this potential or transfer it from one to another species, will be essential (Wollenweber et al., 2005; Den Herder et al., 2010; Kong et al., 2014). However, to achieve this we will need a detailed, mechanistic understanding of the basic developmental processes underlying root system morphogenesis as well as how different environmental conditions impinge on this process.

The branched architecture of plant root systems arises from the continuous formation of additional, new roots. In so-called fibrous root systems, found in the majority of monocot plant species, the original embryonic root loses importance, and new adventitious roots arise from non-root tissues (Atkinson et al., 2014) forming many parallel roots. In contrast, in the dicot tap root system, the embryonic root develops into a persistent main root along which new lateral roots (LRs) are formed, which subsequently reiterate this process, leading to a highly branched root system (Osmont et al., 2007; Bellini et al., 2014). In this review, we focus on the process of LR formation that governs branching in tap root systems, which has been studied in detail in the model plant species *Arabidopsis thaliana*.

In *Arabidopsis*, LRs originate from the pericycle tissue layer that overlays the central vasculature and progress through a well-defined sequence of developmental stages (Figure 1A and 1D) (Malamy and Benfey, 1997). Lateral root formation starts with a process called priming, which prepatterns subsets of pericycle cells to become competent for future LR formation. Priming

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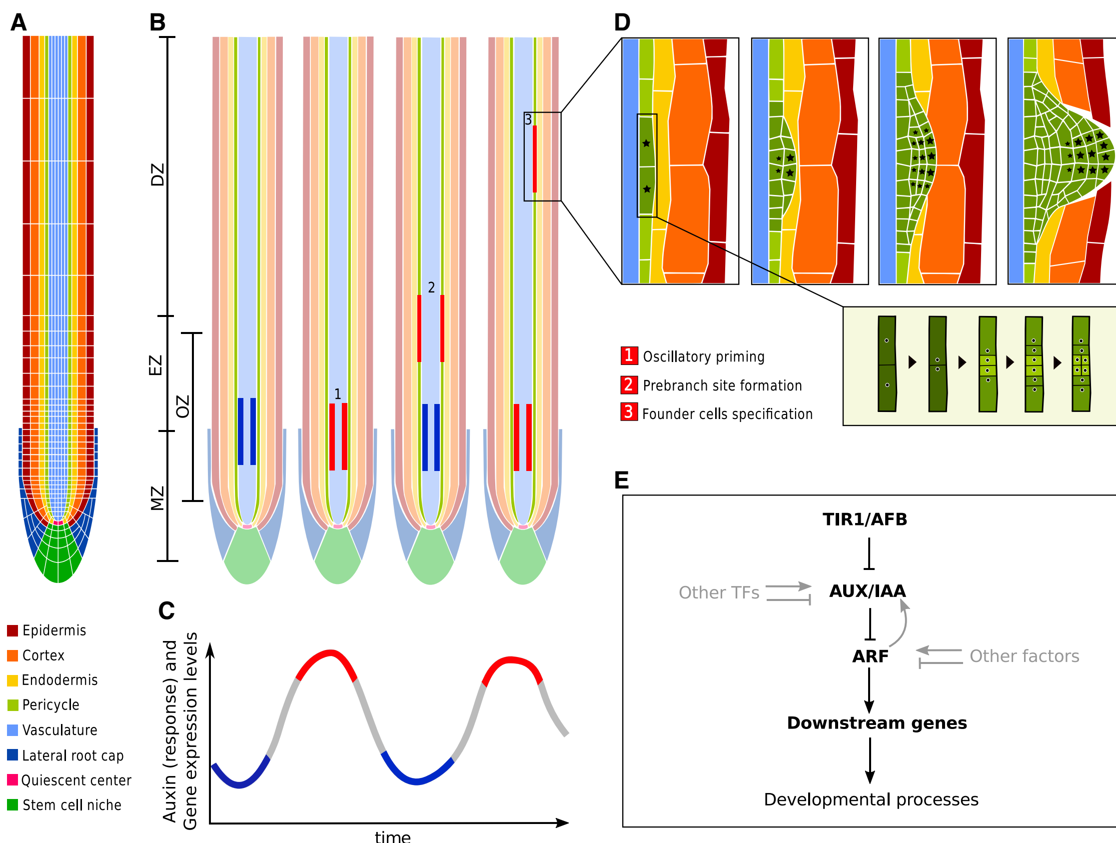

Figure 1. Schematic Overview of Lateral Root Development.
(A) Idealized anatomy of the *Arabidopsis* root tip with the distinct cell and tissue types in different colors.
(B) Schematized depiction of the lateral root priming process, with oscillations of auxin (response) and gene expression in the basal meristem (blue, low levels; red, high levels), stable memorization of the high phase of the oscillation (1) into prebranch sites (2) and subsequently founder cells (3). EZ, elongation zone; DZ, differentiation zone; MZ, meristematic zone; OZ, oscillation zone.
(C) Temporal dynamics of auxin (response) and gene expression in the basal meristem showing oscillations, with low and high levels indicated as in **(B)**.
(D) Stages of lateral root development, from left to right, founder cell identity establishment, initiation, primordium formation, and emergence. Inset shows the nuclear migration and asymmetric divisions typical of lateral root initiation. Stars indicate auxin accumulation.
(E) Layout of the canonical *TIR1/AFB* auxin signaling pathway through which auxin affects gene expression. In light gray, additional regulatory interactions often found in specific auxin signaling modules.

occurs in the shootward end of the meristem (Figure 1B-1) and involves periodic oscillations in auxin (response) and gene expression (Figure 1C) (De Smet et al., 2007; Moreno-Risueno et al., 2010; Xuan et al., 2015, 2016). In case of successful priming, these temporal elevations in auxin (response) and gene expression become transduced into domains of stable high auxin signaling, called prebranch sites (Figure 1B-2). Growth-induced displacement subsequently generates a spatially repetitive pattern of prebranch sites along the root (Moreno-Risueno et al., 2010; Xuan et al., 2015, 2016). Next, prebranch sites develop into so-called lateral root founder cells (LRFCs) (Figure 1B-3), which upon undergoing asymmetric divisions, initiate the actual LR formation process. During subsequent development, LR primordia first penetrate the overlaying endodermal tissue layer, after which cortical and epidermal cells are pushed aside and the primordium emerges (Figure 1D). Next, the meristem of the new LR becomes activated and the LR starts to elongate, eventually recapitulating the pattern of developmental zones present in the main root. Importantly, the plant root system is highly plastic, enabling it to adapt the extent and location of root branching to environmental conditions (Krouk et al., 2010; Mounier et al., 2014; Orman-Ligeza et al., 2018; van Gelderen et al., 2018). This plasticity arises through environmental conditions having an impact on the probabilities of priming to lead to prebranch sites, of founder cells developing into primordia, and of primordia producing emerged LRs.

In this review, we discuss the current knowledge on LR development from a systems biology perspective. Systems biology aims to provide an integrated mechanistic explanation of how interactions between genes, hormones, mechanical forces, and cellular and tissue level processes together give rise to the temporal dynamics and spatial patterns characterizing the biological phenomenon of interest (Noble, 2006). The combination of experimental approaches with modeling has proven to be of great importance for achieving such an integrated understanding. In the current review, we devote specific attention to the insights on LR development that have been gained by complementing experiments with computational

modeling studies simulating the dynamics of the biological processes involved. In addition, we focus on questions regarding "the connecting of the dots" and discuss what is currently known or can be hypothesized about how LR development is transduced from one stage to the next, as well as how the different factors implicated in the spacing of LRs may together form a patterning mechanism.

It is this integration between factors, modules and stages that needs to be further unraveled to move beyond the current descriptions of system subparts and individual stages and move on to a fully integrated systems-level understanding of LR formation. To achieve this, new models capable of simulating the dynamic morphological, mechanical, hydrostatic, and regulatory transitions between different stages of LR development will need to be developed.

TEMPORAL SEQUENCE OF LATERAL ROOT DEVELOPMENT

Auxin signaling plays a major role in all stages of LR development. Often, this involves auxin-driven changes in gene expression. The canonical pathway for auxin-dependent gene regulation involves the auxin sensitive *TRANSPORT INHIBITOR RESISTANT 1/AUXIN SIGNALING F-BOX* (*TIR1/AFB*) receptor, which upon binding with auxin targets the *AUXIN/INDOLE ACETIC ACID* (*Aux/IAA*) repressors for degradation, thereby freeing the auxin response factors (*ARF*s) to become transcriptional activators inducing expression of downstream genes (Figure 1E) (Gray et al., 2001; Zenser et al., 2001; Dharmasiri and Estelle, 2002; Dharmasiri et al., 2005; Kepinski and Leyser, 2005).

In the model plant species *Arabidopsis*, a total of six distinctive *TIR1/AFB* auxin receptors (Mockaitis and Estelle, 2008), 29 *Aux/IAA* repressors, and 23 *ARF*s (Liscum and Reed, 2002; Goh et al., 2012) have been identified. Several *Aux/IAA-ARF* combinations, which we refer to as auxin signaling modules, have been found to be involved in LR development. Although more modules may eventually be discovered, it is currently accepted that four modules are involved in different stages of LR development (Figure 2A) (Lavenus et al., 2013).

Lateral Root Priming

The repetitive, oscillatory prepatterning of lateral root forming sites was first discovered by the Beeckman group. It was found that a priming signal consisting of an elevated auxin response was repeatedly generated in the protoxylem files of the basal meristem and was subsequently transduced to the overlaying pericycle cells (De Smet et al., 2007). Initially, gravitropism-dependent changes in root tip auxin patterns were held responsible for the repeated elevation in auxin levels, but later studies showed that the repeated nature of priming is not caused, but can be modulated by gravistimulation (Moreno-Risueno et al., 2010; Kircher and Schopfer, 2016; Xuan et al., 2016). Next, it was found that in addition to oscillations in auxin response, oscillations in many genetic factors were observed (Moreno-Risueno et al., 2010). The auxin response factor *ARF7* was found to play a critical role in the oscillations; in *arf7* mutants the regular spacing of the prebranch sites generated by priming is found to be severely disturbed. In addition, given that not all auxin responsive factors were found to oscillate, it was postulated that rather than auxin concentration levels oscillating, a genetic oscillator similar to the vertebrate somitogenesis clock was driving the oscillations (Moreno-Risueno et al., 2010). Thus far, no experimental support for such a genetic oscillator has been found. A few modeling studies, simulating root tip auxin dynamics in a single cell or one-dimensional strand of cells, suggested that either the auxin signaling system itself (Middleton et al., 2010) (Table 1) or combined with how it intersects with cytokinin (CK) signaling (Muraro et al., 2011, 2013) (Table 1) could generate oscillations in auxin levels. However, parameter settings necessary to generate oscillatory dynamics were inconsistent with experimental data. More recently, a strong spatiotemporal correlation between LR cap apoptosis and pericycle priming was demonstrated (Xuan et al., 2016). These data led to the hypothesis that repeated LR cap apoptosis, via cells undergoing apoptosis releasing their auxin into the neighboring epidermal cells and subsequently being transported to the vasculature, drives pericycle priming (Xuan et al., 2016). A computational model of auxin dynamics in a two-dimensional static representation of the plant root tip simulating this scenario resulted in a modest 11% increase in pericycle auxin levels following lateral root cap apoptosis (Xuan et al., 2016) (Table 1). This raises the question of whether this mechanism could provide a sufficiently strong and robust priming signal. Based on the observed correlation between root cap growth dynamics and apoptosis, as well as the fact that vasculature auxin signaling is necessary for LR priming (De Smet et al., 2007) and synthesis of an auxin precursor in the LR cap strongly determines priming amplitude (Xuan et al., 2015), we recently developed a dynamic, two-dimensional root tip model investigating the interplay between root growth and auxin reflux dynamics in the context of priming (Van den Berg and ten Tusscher, 2018). The model builds on our earlier developed models (Mähönen et al., 2014; van den Berg et al., 2016), incorporating details of root tip auxin transport dynamics, root developmental zonation, and root growth dynamics. Our computational study suggested that priming with auxin maxima increasing auxin levels by 40% or more automatically arise as an emergent property of root growth dynamics and reflux loop properties (Van den Berg and ten Tusscher, 2018). Briefly, the root tip reflux loop generates an auxin loading zone at the shootward end of the meristem. In addition, root growth dynamics generate periodic variations in the sizes of cells arriving at this zone. Combined, this gives rise to the periodic arrival of large cells with elevated auxin uptake capacity due to their larger surface to volume ratio, thus resulting in periodic auxin oscillations. The model predicts that while priming frequency predominantly depends on cell division frequency in the meristem, LR density additionally depends on meristem size with larger meristem resulting in increased interlateral root spacing. These model predictions agree well with available experimental data; for example, the observation that application of D15, an inhibitor of carotenoid cleavage dioxygenases, results in both a substantial reduction of cell sizes as well as reduced priming amplitude and hence the number of LRs actually formed (Dickinson et al., 2018), the lower number of LRs formed in *cycd4:1* mutants showing reduced pericycle cell division activity (Nieuwland

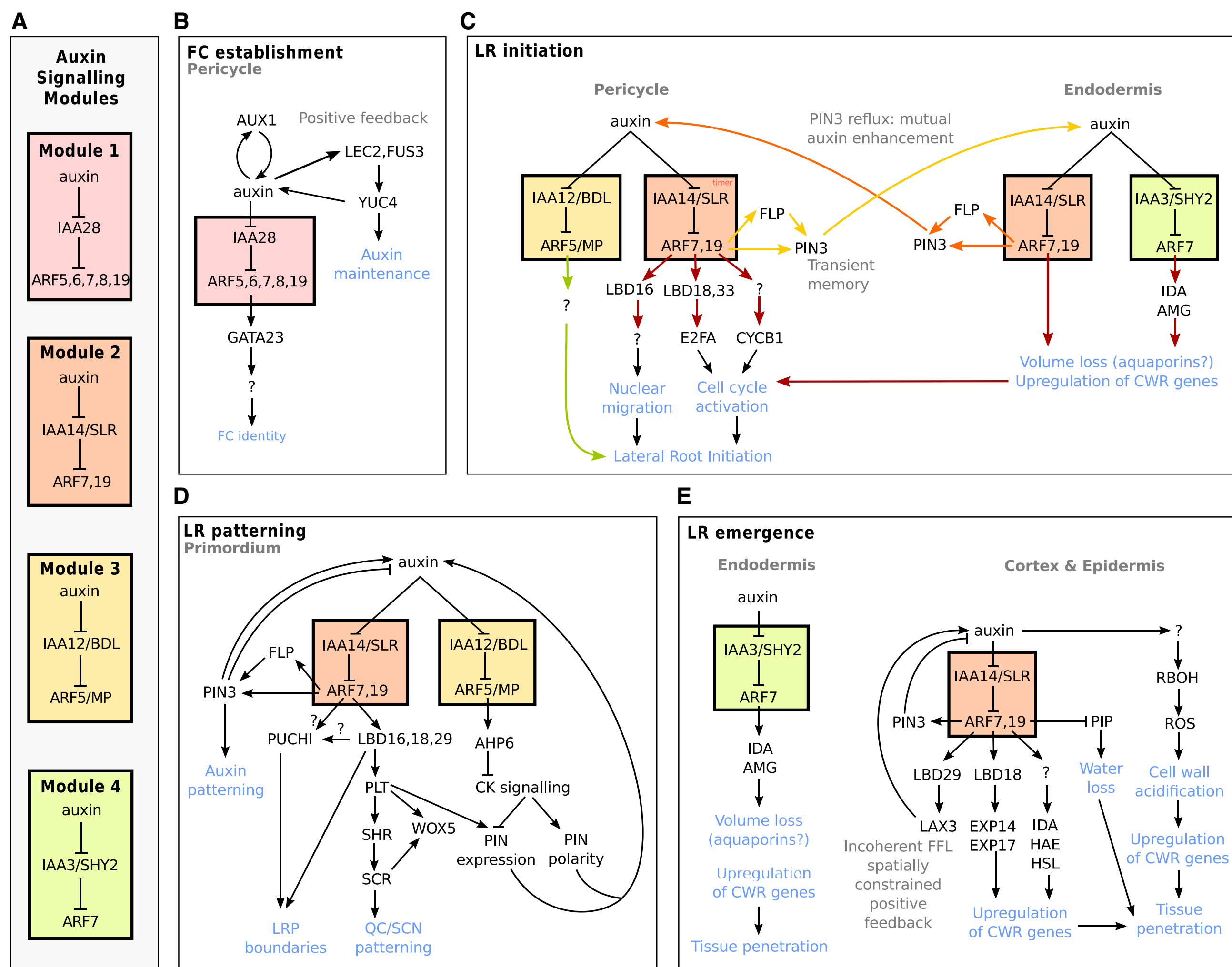

Figure 2. Overview of the Auxin Signaling Regulatory Modules Involved in Lateral Root Development.

(A) Layout of auxin signaling modules 1–4, with their specific *Aux/IAA* and *ARF*s, found to be involved in lateral root development.

(B) Pericycle activation of module 1, together with *AUX1* and the *LEC2*, *FUS3*, *YUC4* loop are involved in founder cell identity establishment. The auxin-inducible *AUX1*, and *LEC2*, *FUS3*, *YUC4* loop give rise to positive feedback that elevates and maintains auxin levels.

(C) Lateral root initiation involves the activation of modules 2 and 3 in the pericycle founder cells, and of modules 2 and 4 in the overlaying endodermal cells. First, the pericycle founder cells activate module 2, causing *PIN3* driven export of auxin into the endodermis (yellow arrows). The resulting auxin increase in the endodermis now also activates module 2 there, thus giving rise to a *PIN3* reflux loop (orange arrows). As a consequence, pericycle and endodermal auxin levels rise. This results in further activation of module 2 (red arrows) in the pericycle as well as module 3 (green arrows), which together control nuclear migration and asymmetric divisions. At the same time, in the endodermis module 4 becomes active (red arrows), driving the volume loss and cell wall remodeling.

(D) Further divisions and patterning of the growing primordium continue to involve modules 2 and 3. Module 2 controls *PUCHI*, involved in patterning LRP boundaries, and the *PLETHORA* transcription factors that are critical for internal patterning of the primordium. Module 3 represses CK signaling through *AHP6*, setting up a low CK signaling domain where auxin levels are high, thereby preventing CK-induced downregulation of *PIN* levels in this domain. At the same time, the moderate CK levels inside the high auxin domain, and the higher CK levels outside this domain enable the polarization of *PIN* toward the high auxin domain, enhancing and stabilizing this domain.

(E) Lateral root emergence involves the passage of first the endodermis, and after this the cortex and epidermis. Passage of the endodermis involves auxin signaling module 4, previously involved in initiation and now likely activated to a larger extent to enhance volume loss and cell wall remodeling necessary for the primordium to penetrate the endodermis. Passage of the cortex and epidermis involves the activation of module 2. In these tissues, module 2 causes the sequential activation of first *PIN3* and then *LAX3*, which enables a localized increase in auxin levels that subsequently enables the activation of expansins and cell wall remodeling enzymes. In parallel, ROS signaling induces cell wall acidification, while upregulation of aquaporins changes turgor in overlaying tissues. Combined, this enables the separation and pushing aside of cortical and epidermal cells by the primordium.

et al., 2009), as well as the inverse correlation observed for meristem size and LR density for a wide variety of conditions (Gruber et al., 2013). Still the proposed mechanism remains to be experimentally validated.

Founder Cell Identity Establishment

The first event following a successful priming stimulus is the formation of a stable prebranch site, characterized by a persistent high auxin response (Moreno-Risueno et al., 2010). Due to the

Article	Spatial context Spatial formalism Growth	Modeled dynamics Model formalism	Fitted to/estimated from
Priming			
Middleton et al., 2010	Single cell	Auxin, TIR1/SCF, IAA/AUX, ARF: ODEs	Known interactions between molecular players
Muraro et al., 2011	Single cell	Auxin, SCF1/TIR, AUX/IAA, ARF, PIN, CK, AHK, ARR, CK response: ODEs	Known interactions between molecular players
Muraro et al., 2013	One 1D row of cells Non-growing	Auxin, AUX/IAA, ARF, PIN, CK: ODEs	Known interactions between molecular players
Xuan et al., 2016	Realistic 2D root tip Vertex-based cells Non-growing	Auxin: ODE	Root tip tissue topology, PIN patterns and polarity, AUX/LAX patterns, root developmental zones, cell sizes and shapes in different root zones
Van den Berg and ten Tusscher, 2018	Realistic 2D root tip Grid-based cells Growing	Auxin: PDE AUX1: ODE	Root tip tissue topology, PIN patterns and polarity, AUX/LAX patterns, auxin-dependent AUX1 expression, root developmental zones, cell sizes and shapes in different root zones, cell cycle duration, cell expansion duration
Founder cell establishment, initiation, and sidedness			
Laskowski et al., 2008	Squarish 2D root tip Grid-based cells Non-growing	Auxin: PDE AUX1: ODE	PIN patterns and polarity, auxin-dependent AUX1 expression, root developmental zones, cell sizes in different root zones
El-Showk et al., 2015	Realistic 2D cross section Grid-based cells Non-growing	Auxin, CK: PDEs AUX1, PIN3, PIN1, PIN7: ODEs	Root cross section tissue topology, PIN patterns and polarity, auxin dependence of AUX1
Chen et al., 2015	Single cell	Auxin, ARF7, FLP, PIN3: ODEs	FLP dependence on ARF7 and PIN3 dependence on ARF7 and FLP
LRP growth and development			
Von Wangenheim et al. 2016	Two-dimensional cross section of LRP Growing	Tissue growth and cell divisions Bezier curves for tissue growth, vertex-based cell modeling	Growth profile of outer boundary LRP
LR emergence			
Péret et al., 2013	Realistic 2D cross section Vertex-based Non-growing	Auxin, PIN3, LAX3: ODEs	Root cross section tissue topology, cortical, auxin-dependent LAX3, and PIN3 expression
Péret et al., 2012	Simplified compartment-based cross section Vertex-based Growing	Water transport, pressure, deformation: ODEs	Size of and pressures in different compartments, water permeabilities, rate of osmotic pressure increase fitted to obtain 28 h emergence time

Table 1. Overview of the Properties of the Computational Model Studies Discussed in the Main Text.
Models are given in the order they are discussed in the main text, and are grouped based on the stage of LR development modeled. 1D, one-dimensional; 2D, two-dimensional; ODE, ordinary differential equation; PDE, partial differential equation. Importantly, models are mostly based on qualitative data on tissue layout, expression patterns, and known interactions. Few data are available on rate constants or affinity constants. This limitation in data availability is generally handled by checking whether model outcomes are robust to changes in the values of parameters for which no data are available. Parameters for auxin transport in most models can be traced back to the pioneering models by Mitchison (Mitchison, 1980) and Kramer (Kramer, 2002) (e.g. Swarup et al., 2008).

limited spatial precision of auxin reporters, it is currently unclear whether stable prebranch sites correspond one to one to cells obtaining LRFC identity, or rather that only a subset of cells in a stable prebranch site subsequently obtains founder cell identity (Du and Scheres, 2018). Experiments have shown that after the priming-induced auxin (response) peak, auxin (response) levels initially decline (Xuan et al., 2015). Only if priming amplitude is sufficiently high, a secondary rise and subsequent persistent maintenance of auxin levels leading to stable prebranch site formation occurs (Xuan et al., 2015). These data imply that founder cell specification requires active accumulation of auxin. Consistent with this, the auxin-inducible (Gazzarrini et al., 2004;

Horstman et al., 2017) *LEAFY COTYLEDON2* (*LEC2*) and *FUSCA3* (*FUS3*) factors inducing the *YUCCA4* (*YUC4*) auxin biosynthesis gene are expressed in founder cells (Tang et al., 2017). In addition, experiments show that while priming in the basal meristem occurs at both xylem poles, LRs are formed at only one of the two poles. Modeling studies have played a critical role in unraveling the mechanism underlying the sidedness of LR formation (Laskowski et al., 2008; El-Showk et al., 2015).

The auxin-inducible *AUXIN RESISTANT 1* (*AUX1*) auxin importer has been found to be expressed from the earliest stages of LR development (Marchant et al., 2002). By combining experiments with a computational model of auxin dynamics in a simplified, static two-dimensional root model, it was demonstrated that the auxin inducibility of *AUX1* generates a positive feedback loop. This feedback loop enables the amplification of modest auxin increases, ensuring the generation and maintenance of robust auxin maxima (Laskowski et al., 2008) (Table 1). A subsequent modeling study simulating auxin–cytokinin interactions in a two-dimensional cross section of the root suggested how this auxin-*AUX1* positive feedback also enables the amplification of differences between the two vascular xylem poles, ensuring the formation of a single-sided auxin maximum (El-Showk et al., 2015) (Table 1). To the authors knowledge, the precise pathway leading from auxin to *AUX1* upregulation and whether it overlaps with the auxin signaling modules that have thus far been implicated in LR development, has so far not been elucidated. Still, specification of founder cell identity involves a first auxin signaling module, which is centered around *IAA28* (Figure 2B). Downstream of this are *ARF5*, *ARF6*, *ARF7*, *ARF8*, and *ARF19* (De Rybel et al., 2010). The most well-known downstream target of this pathway, *GATA23*, is generally used as the earliest indicator of founder cell identity (De Rybel et al., 2010), yet a clear function has thus far not been identified. Apart from experiments aimed at directly establishing network interactions, insights may also be gained from studies combining large-scale transcriptomics analyses with network inference algorithms.

Lateral Root Initiation

The next stage in LR development, called initiation, involves a series of events (Figure 2C). Within the LRFCs, first migration of the nuclei to the cell end neighboring the shared cell wall occurs, after which multiple asymmetric divisions ensue (De Smet et al., 2007). For these events to occur, first the overlaying endodermal tissue must lose volume and shrink to lift a mechanical constraint that prevents cell cycle initiation in the pericycle (Vermeer et al., 2014; Marhavý et al., 2016). Pericycle nuclear migration and asymmetric divisions as well as an endodermal accommodation mechanism are auxin dependent (De Rybel et al., 2010; Berckmans et al., 2011; Vermeer et al., 2014) and require coordinated auxin signaling between the two tissue layers. Auxin-dependent LR initiation processes in the pericycle occur downstream of a second auxin signaling module, which starts with the repression of *SOLITARY ROOT* (*SLR*)/*IAA14*, degradation of which to a large extent next derepresses the same *ARF*s as those employed in the previous module. Chen et al. (2015) demonstrated that, as part of this module, the auxin transporter *PIN-FORMED 3* (*PIN3*) was induced both directly by *ARF7*, as well as indirectly via the intermediate *FOUR LIPS* (*FLP*/*MYB124*) factor. Using a computational model simulating auxin, *ARF7*, *FLP*, and *PIN3* dynamics of an individual cell, the authors subsequently showed that the thus formed so-called coherent feed forward motif enables a prolonged maintenance of *PIN3* induction in response to a transient auxin increase, lending a temporal memory to the system (Table 1). This *PIN3* induction is essential for ensuring an auxin maximum inside the founder cells and later forming primordium as well as into the overlaying endodermal tissue. In the endodermis, a transient expression of *PIN3*, polarized toward the pericycle founder cells, has been shown to establish an auxin reflux loop between the pericycle and endodermis that contributes to auxin accumulation and LR initiation (Marhavý et al., 2013).

We speculate that it is the module2-*PIN3*-mediated auxin export from the pericycle to the endodermis (Figure 2C, yellow arrows) that transiently activates a similar module2-*PIN3* response in the endodermis to generate this auxin reflux and elevate auxin levels in both pericycle and endodermis (Figure 2C, orange arrows). Once auxin levels in the endodermis have been elevated, volume loss and shrinkage occur downstream of the auxin-induced *SHORT HYPOCOTYL2* (*SHY2*) factor (Vermeer et al., 2014; Vermeer and Geldner, 2015), considered the central component of an auxin signaling module generally referred to as module 4 (Lavenus et al., 2013). Also, downstream of the auxin module 2 active in the pericycle founder cells are *LATERAL ORGAN BOUNDARIES-DOMAIN 16* (*LBD16*), *LBD18*, and *LBD33* (Figure 2C, red arrows), which are required for nuclear migration and the asymmetric divisions that are the hallmark of LR initiation, respectively (Berckmans et al., 2011; Goh et al., 2012). Finally, *CYCLIN B1* (*CYCB1*), necessary for cell cycle activation, is induced downstream of module 2 (Vanneste et al., 2005; Okushima et al., 2007) as well as endodermal accommodation (Marhavý et al., 2016), thus ensuring coordination between pericycle and endodermal events. In addition to module 2, an auxin signaling module consisting of *IAA12*/BODENLOS (*BDL*) and *ARF5*/*MONOPTEROS* (*MP*), generally referred to as module 3 is also involved in LR initiation (Figure 2C, green arrows) (De Smet et al., 2010). This module is induced somewhat later than module 3, yet in *slr* mutants can at least partly rescue LR initiation (De Smet et al., 2010). Through which downstream targets it controls nuclear migration and asymmetric division remains to be established.

Lateral Root Development

After initiation, cell divisions continue to give rise to a growing lateral root primordium (LRP). Although divisions are restricted to the more central cells, the timing and orientation of divisions is non-stereotypical (Lucas et al., 2013; Von Wangenheim et al., 2016). Experiments have demonstrated that the mechanical constraints imposed by the overlaying tissues ensure that primordia are channelled into their typical dome shape, with control of cell division playing a less important role (Lucas et al., 2013). Still, while this explains the typical dome shape, it does not yet explain how the organized layered structure of an LRP can arise despite non-stereotypical cell division patterns. Using a two-dimensional computational model of LRP growth, in which different growth profiles (homogeneous, basally

dominated) and cellular division rules (random, geometric) were tested, it was shown that a simple, probabilistic shortest-path division rule is sufficient to spatiotemporally self-organize LRP division patterns (Von Wangenheim et al., 2016) (Table 1). The resulting pattern consists of a regular alternation of division plane orientations within individual cells with periclinal divisions occurring earlier in outer tissue layers that together generate the typical layered LRP tissue layout (Von Wangenheim et al., 2016). As the primordium thus grows and develops, the initially diffuse, broad auxin pattern defining the LRFCs is transformed into a distinctive auxin gradient with its maximum residing at the LRP tip (Benkova et al., 2003). In parallel with these changes in auxin patterns, the expression patterns of the auxin-exporting PIN proteins undergo substantial changes, with *PIN1* becoming more and more restricted to the vasculature, *PIN3* becoming restricted to the region close to the quiescent center (QC) and more distal vasculature, and *PIN2* arising only after primordium emergence (Benkova et al., 2003). Differences between *PIN* types in how expression and degradation depend on auxin levels have been suggested to help pattern *PIN* domains (Mironova et al., 2012), and additional regulatory differences are likely to exist. In addition to changes in expression domain, also *PIN* polarity patterns change, with *PIN1* changing from a predominantly inward oriented to a tipward oriented polarity (Omelyanchuk et al., 2016) and *PIN3* likely undergoing similar changes.

The third auxin signaling module that comes into play during LR organogenesis is thought to play an important role in this canalization of auxin flux to the newly forming LRP tip. The module consists of the auxin-dependent degradation of *BDL/IAA12*, which derepresses *MP/ARF5* (Figure 2D) (De Smet et al., 2010). Given the known relation between the *BDL/MP* module and *ARABIDOPSIS HISTIDINE PHOSPHOTRANSFER PROTEIN6* (*AHP6*) in the vasculature (Ohashi-Ito et al., 2014), this module is likely responsible for the observed expression of *AHP6* (Moreira et al., 2013). *AHP6* represses CK signaling, thereby preventing CK from repressing LRP *PIN* levels (Laplaze et al., 2007; Moreira et al., 2013). In addition, CK has been found to result in the removal of *PIN1* from specific cell membranes (Marhavý et al., 2011), resulting in the repolarization of *PIN1* toward the tip of the newly forming primordium (Marhavý et al., 2014). In several reviews, these interactions have been summarized as *AHP6* directing *PIN1* polarization (see e.g. Taylor-Teeples et al., 2016), yet it is important to keep in mind that *AHP6* represses CK signaling and hence prevents *PIN* expression from being strongly downregulated, while remaining CK signaling polarizes *PIN1*. Together, this results in a *PIN1*-mediated auxin flow directed to the primordium tip. This auxin flux is essential for the patterning of a proper auxin maximum, necessary to establish the QC and stem cell niche (Benkova et al., 2003).

In parallel, and both up- and downstream of auxin and *PIN* patterning, the continued activity of auxin response module 2 leads to the induction of the *PLT* genes (Feng et al., 2012), critical for proper LRP patterning (Du and Scheres, 2017). The *PLT* genes were demonstrated to control the proper expression of the auxin transporters *PIN1* and *PIN3*, as well as the *SHR*, *SCR*, and *WOX5* transcription factor genes. In *plt* triple mutants, a proper auxin maximum, normal transcription factor pattern or a QC and meristem are not formed, resulting in abnormally shaped, arrested primordia (Du and Scheres, 2017). How the interactions between auxin, cytokinin, major transcription factors such as the PLTs and the *PIN*s together pattern the root tip auxin reflux loop in a self-organized manner remains to be elucidated. In addition to inducing the *PLT*s, module 2 also controls LRP patterning by inducing the *PUCHI* gene that is involved in defining primordium boundaries (Hirota et al., 2007; Kang et al., 2013).

Lateral Root Emergence

For LRs to emerge from inside the main root, the overlaying endodermal, cortical, and epidermal tissue layers have to be passed. The endodermis is a highly specialized tissue, containing the lignified, supracellular Casparian strip that seals the inner root tissues from the outside by tightly binding endodermal cell membranes to one another (Vermeer et al., 2014). As a consequence, penetration of the endodermis is fundamentally different from the subsequent penetration of cortical and epidermal tissue layers (Stoeckle et al., 2018). In order to preserve the apoplastic diffusion barrier formed by the Casparian strip, penetration of the endodermis occurs through endodermal volume loss and flattening, leading to the fusion of endodermal radial membrane faces to generate an opening through which the primordium can pass (Vermeer et al., 2014). In contrast, passage of the cortex and epidermis, while also involving volume and turgor loss (Péret et al., 2009, 2013), strongly depends on cell wall remodeling (CWR), enabling the pushing LRP to cause the overlaying cells to detach from one another and move to the sides (Laskowski et al., 2006; Swarup et al., 2008; Péret et al., 2009; Kumpf et al., 2013).

Passage of the endodermis, like the earlier endodermal accommodation process, involves auxin signaling module 4, centered around *SHY2/IAA3*, and *ARF7* as well as possibly other *ARF*s to induce endodermal volume loss and shape changes (Figure 2E) (Vermeer et al., 2014). After passing of the endodermis, module 2 is activated in the overlaying cortical and epidermal tissue layers to guide the next steps of LR emergence (Figure 2E). Within this different tissue context, while deploying the same *IAA* and *ARF*s as in the primordium cells, a different set of downstream targets become activated. Activation of the *LIKE-AUXIN3* (*LAX3*) auxin importer plays a major role in elevating cortical and epidermal auxin levels and inducing the necessary CWR (Swarup et al., 2008). To investigate this further, a model was developed simulating auxin dynamics and *PIN3* and *LAX3* expression in a two-dimensional cross section of the root at the location of a developing LRP. It was shown that in order for the *LAX3*-mediated auxin elevation to occur in a localized manner in only those cells that are overlaying the primordium, the additional and earlier activation of an auxin exporter is required (Péret et al., 2013) (Table 1). This auxin exporter was subsequently identified to be *PIN3* (Péret et al., 2013). The earlier activation of *PIN3* relative to *LAX3* is consistent with the fact that *PIN3* activation occurs directly downstream of *ARF7* and *ARF19* (Chen et al., 2015), whereas *LAX3* is induced downstream of *LBD29* (Okushima et al., 2007; Porco et al., 2016), which itself is downstream of *ARF7* and *ARF19*.

LAX3 expression has been shown to be essential for the activation of a series of CWR enzymes (Swarup et al., 2008; Kim and Lee,

2013; Kumpf et al., 2013; Lee and Kim, 2013; Lee et al., 2014, 2015). While in many schematic depictions of LR emergence, this has led to the induction of these CWR enzymes being depicted as being downstream of *LAX3* (see e.g. Du and Scheres, 2018), the expansins involved, *INFLORESCENCE DEFICIENT IN ABSCISSION* (*IDA*), *HAESA* (*HAE*), and *HAESA-LIKE2* (*HSL*), are in fact auxin dependent and downstream of the same *ARF7* and *ARF19* that are upstream of *LAX3* and hence genetically part of parallel pathways. Thus, the dependence of CWR genes on *LAX3* expression likely indicates that induction of CWR enzymes requires higher auxin and hence *ARF7* and *ARF19* levels than the induction of *LAX3* itself (Swarup et al., 2008). Indeed, it has been suggested that the auxin dependence of *LAX3* creates a bistable switch, and it is the switching to the high auxin high *LAX3* state that then induces the CWR genes. In addition to the induction of CWR enzymes, auxin also induces the expression of *RESPIRATORY BURST OXIDASE HOMOLOGS* (*RBOH*), leading to production of reactive oxygen species (ROS) and cell wall acidification, which further contributes to the degradation of cell walls (Orman-Ligeza et al., 2016). In addition to CWR, also changes in turgor of the primordium and its overlaying tissues, with the LRP gaining and the overlaying tissues losing water, contribute to LR emergence. Péret et al. (2012) demonstrated that auxin causes a spatially restricted repression of water-transporting aquaporin channels (PIPs). Using a simple compartment model of water transport between the vasculature, primordium, and overlaying tissues, it was shown that the resulting changes in water transport enable volume loss in overlaying tissues as well as build-up of turgor inside the primordium (Péret et al., 2012) (Table 1). It is the combined weakening of cell walls and the increase in turgor of the primordium relative to its overlaying tissues that enables LR emergence.

Specificity

Specificity of auxin action arises from different *Aux/IAA*s having different auxin binding affinities, enabling them to respond to different auxin levels (Villalobos et al., 2012; Shimizu-Mitao and Kakimoto, 2014). In addition, tissue-specific expression of *Aux/IAA* and *ARF*s results in different *Aux/IAA-ARF* combinations in different contexts (Weijers et al., 2005; Rademacher et al., 2011). Intriguingly, while all four modules involved in lateral root development are characterized by a unique *Aux/IAA* repressor, as well as distinct sets of downstream target genes, the intermediate *ARF*s show a large degree of overlap between the different modules. This is particularly true for *ARF7*, which occurs in modules 1, 2, and 4. Thus, the question is how *Aux/IAA* specificity can be maintained despite signals going through the same downstream *ARF*s. A potential explanation could be that downstream targets of later-acting modules require higher levels of the same *ARF*s for their induction. In that case, either the combined degradation of *Aux/IAA*s of both earlier and later modules, or a larger effect of the degradation of later *Aux/IAA*s on free *ARF* levels, would ensure the later induction of these target genes.

In addition to the occurrence of *ARF7* in at least three modules, module 2 itself is involved in a series of developmental events that involve different downstream targets. In this case, both *ARF* and *Aux/IAA* factors are shared, even more strongly raising the question of how specificity can be achieved. Part of the specificity here may arise from the different tissue contexts in which *ARF7* is deployed, which may result in the presence of different co-factors or differential availability of downstream targets (Boer et al., 2014).

PROGRESSION BETWEEN STAGES

While substantial experimental and modeling effort has been devoted to unraveling the regulatory logic, dynamics, and patterning of individual developmental stages, far less attention has been devoted to how one developmental stage or process leads to the next. In addition to the question of how a transition between stages is set into motion, important questions are how such transitions are ensured to occur in a robust, coherent manner.

Theoretically, several scenarios can be envisioned. First, the temporal sequence of events could arise from the changing tissue context that LR forming sites find themselves in as the development of surrounding tissues progresses over time. In this scenario, the gradual change in tissue context would sequentially trigger the different auxin modules and associated genes involved as well as ensure that these transitions cannot be reverted. Given that LR priming results in an acropetal pattern, yet subsequent LR development may break this acropetal sequence with further developed LRs potentially occurring rootward of early-stage LR primordia (Charlton, 1975; MacLeod, 1990; Dubrovsky et al., 2000, 2006), this scenario appears unlikely to fully explain all sequential steps in LR development. Still, the fact that priming occurs at the shootward end of the meristem, yet the earliest signs of initiating LRs are only visible beyond the elongation zone, may suggest that the transition from founder cells to lateral root initiation may indeed depend on developmental context (Dubrovsky et al., 2011). A zonation-dependent drop in CK signaling has been suggested as a candidate for constituting this developmental gating (Bielach et al., 2012). Consistent with the idea of CK as a developmental gate, CK signaling has been found to repress LR *PIN* expression (Laplaze et al., 2007) and *AUX1* expression (Street et al., 2016), as well as inhibit the cell cycle activity necessary for LR initiation (Li et al., 2006). It is currently unclear whether the repressive effect of CK on the cell cycle is simply through repressing auxin levels or also through more directly affecting cell cycle genes. Given the previously mentioned auxin-dependent induction of *AUX1* and the induction of *YUC4* downstream of *LEC2/FUS3*, the following scenario can be envisioned. First, priming initially results in a modest induction of *AUX1* and consequently also a modest induction of *LEC2/FUS3* and *YUC4*, enabling the re-establishment and maintenance of auxin levels after priming. The resulting auxin levels enable the activation of the first auxin signaling module responsible for founder cell identity establishment. Next, as cells move shootward and CK levels drop, *AUX1* levels are able to further increase, enabling also enhanced induction of *LEC2/FUS3/YUC4*. Together this gives rise to a further ramping up of pericycle auxin levels, resulting in the induction of module 2. In addition, the drop in CK levels may potentially lift CK-dependent direct repression on cell cycle activity, enabling module 2 to induce LR initiation. The transition to module 2 is subsequently stably locked in via three synergistic mechanisms. First, module 2 also becomes active in the endodermis, resulting in the *PIN3*-mediated auxin reflux discussed earlier that contributes to

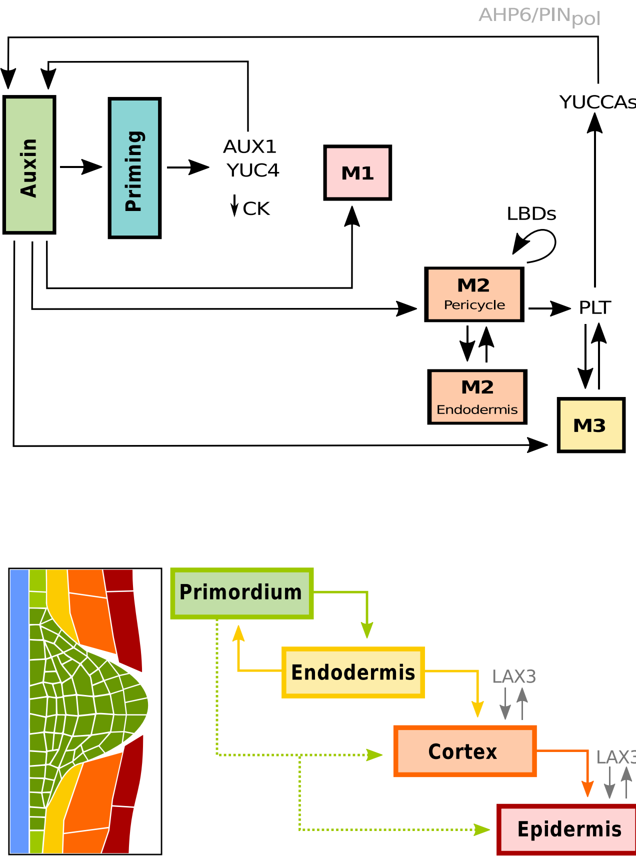

Figure 3. Depiction of the Sequence of Transitions between Stages of Lateral Root Development.

(A) Sequential activation of auxin signaling modules 1, 2, and 3 in the primed pericycle cells during founder cell establishment, initiation, and further development. Successful priming induces *AUX1* and, via *LEC2* and *FUS3*, *YUC4*, thereby re-establishing and maintaining auxin levels, enabling the induction of module 1. As cells progress shootward, cytokinin levels drop, derepressing *AUX1* expression, and thereby enabling a further increase in auxin levels that now results in activation of module 2. Activation of module 2 causes a further increase in auxin levels through the *PIN3*-mediated reflux with the endodermis, as well as *PLT*-dependent induction of *YUCCA* genes. The autoactivation of *LBD* transcription factors further ensures a stable locking in of this stage. Next, the *PLT* expression downstream of module 2 induces *ARF5/MP*, part of module 3, with *ARF5/MP* upregulating *PLT3*, ensuring a robust transitioning to module 3.

(B) Sequential transmission of auxin from the primordium to the endodermis, cortex, and epidermis involves the *PIN3*-mediated export of auxin from the primordium (green arrow), *PIN3* reflux-mediated uptake in the endodermis (upward yellow arrow), *LAX3*-mediated uptake in the cortex and epidermis (gray arrows), and the auxin-induced giving way of overlaying tissues enabling the pericycle to first reach the endodermis, then cortex, and finally the epidermis (dashed green arrows).

elevating auxin levels. Second, downstream of module 2 are the *PLETHORA* transcription factors, which by inducing the auxin biosynthetic *YUCCA* genes, further enhance auxin production (Figure 3A). Finally, the positive feedback loop resulting from the induction of *ARF7* and *ARF19* by *LBD18* (Pandey et al., 2018) ensures the transition to a new stable state.

A second possibility is that the temporal sequence of module activation is inherent to the wiring within and between modules, i.e. that the *ARF*s or downstream genes of a module either through inducing the *ARF*s or repressing the *IAA*, or some other involved repressor of a second module, activate or sensitize this second module and set it into motion. In this scenario, necessary players of later modules may only become (sufficiently) expressed in response to activation by an earlier module. Although this question has not been explicitly investigated, some knowledge may be gleaned from the downstream targets the different auxin modules have. The *PLETHORA* transcription factors 3, 5 and 7 are auxin-inducible downstream of *ARF7/ARF19* and *LBD*s, which are part of module 2 (Feng et al., 2012) and become active during early stages of lateral root development (Du and Scheres, 2017). The *PLETHORA*s have been shown to induce *ARF5/MP* auxin response factor (Santuari et al., 2016), which is central to module 3. Furthermore, given that *ARF5/MP* has been shown to induce *PLT3* (Yamaguchi et al., 2016), a feedback loop arises ensuring a robust and irreversible transition from module 2 to module 3 once a certain *PLT* threshold level has been exceeded. An increase in auxin sensitivity, arising from *BDL/MP*-dependent induction of the CK signaling inhibitor *AHP6* is likely to further contribute to this locking on to the next state.

A final option is that a later module is less auxin sensitive, thus requiring higher auxin levels to become activated, and/or that a later module is active in different tissues and auxin levels first have to become elevated there. In this scenario, the activation of an early module, through its downstream targets, may elevate auxin (signaling) levels in the LRP cells and/or its surroundings such that the second module becomes activated. Measured auxin sensitivities of the involved *Aux/IAA*s, *IAA28*, *IAA14*, *IAA12*, and *IAA3* active in modules 1, 2, 3, and 4 respectively, suggest no significant differences in auxin sensitivity (Shimizu-Mitao and Kakimoto, 2014). Except for possibly the transition from module 1 to 2 discussed above, it is thus unlikely that it is simply an incremental increase in auxin signaling levels that is driving the temporal order of module activities. In contrast, while founder cell identity establishment appears to mainly involve auxin accumulation in the competent pericycle cells, subsequent initiation also requires auxin accumulation in endodermal cells, while in yet later stages, endodermis penetration and passage of the cortex and epidermis also involve auxin accumulation in these tissues. Thus, the sequential activation of first modules 2 and 4 in the endodermis and next of module 2 in the cortex and epidermis appears to involve the spatiotemporally ordered accumulation of auxin in more and more outward tissue layers. We suggest that this "wave" of auxin signaling (Péret et al., 2013) involves a total of five distinct processes. First, sufficient auxin has to accumulate in the primordium for it to result in substantial transport of auxin into the next layer. To maintain elevated auxin levels in a growing primordium and induce elevation of auxin levels in overlaying cells, an increase in overall auxin availability inside the primordium is required. Robust increases in available auxin are achieved via the auxin-dependent activation of auxin biosynthesizing genes downstream of *PLT* (Santuari et al., 2016) as well as the early active *LEC2/FUS3* transcription factors (Tang et al., 2017). Again, this regulatory architecture gives rise to a positive feedback loop that enables the robust transitioning to and locking in of subsequent developmental stages. Combined with the auxin-dependent induction of *PIN3*, the elevated auxin levels enhance the capacity for auxin export into the tissues overlaying the primordium. Next, this transport of auxin first into

the endodermis causes a local auxin increase that induces module 4 driven volume loss and CWR (Vermeer et al., 2014), enabling first endodermal accommodation and later the passage of the primordium through the endodermis. Importantly, passage of the endodermis allows the primordium to now transport auxin directly to the cortex (Figure 3B). Here, an initially modest auxin increase triggers module 2, first switching on *PIN3* and subsequently *LAX3* expression to switch on an incoherent feedback loop, with *PIN3* locally reducing and *LAX3* locally enhancing auxin levels that ensures elevation of auxin levels in a highly localized manner (Péret et al., 2013). These high auxin levels are required to subsequently induce CWR and changes in tissue turgidity, which together enable the LR primordium to pass the cortex and transport auxin to the epidermis where the same sequence of events is now repeated (Figure 3B). Consistent with the idea of a passing wave of auxin, *LAX3* transcription is upregulated under a *PIN3* increase (Péret et al., 2013).

In the above, we identified possible players and interactions explaining the transition from priming to the activation of module 1, the activation of module 3 by module 2, and the activation of modules 4 in the endodermis, and module 2 in the cortex and epidermis from auxin transport of the pericycle into the overlaying tissues. An open question is what drives the activation of module 2 involved in LR initiation in the pericycle. Here, two contributing factors can be envisioned. First, this transition could, like the transition from priming to activation of module 1, also entail the positive feedback arising from auxin-induced *AUX1* and *YUCCA4* expression. A further drop in CK levels as cells move shootward in the differentiation zone may enable a second, further increase in *AUX1* and hence auxin levels, thus activating module 2. In addition, once a minor activation of module 2 has occurred, the *PIN3* reflux between the pericycle and endodermis enhances auxin levels, which could lead to the further activation of module 2 (Figure 3A).

Studies specifically aimed at reconstructing how one developmental stage progresses to the next have thus far been scarce. A notable exception is the study by Lavenus and colleagues, in which large-scale transcriptomics data from a time series of LR development were combined with network inference algorithms to reconstruct network topology and temporal ordering of gene activity (Lavenus et al., 2015). In addition to many positive feedbacks and coherent feedforward loops, in line with the above analysis, which are important for ensuring robust transitions to a next stage, in this study also many negative feedbacks were inferred. Interestingly, the inferred network topology and gene activity ordering suggested that these negative feedbacks represent later-acting genes that inactivate earlier active genes. The importance of auxin-induced repression of gene expression was earlier suggested by a study that elegantly combined a series of different LR transcriptomic datasets into a single compendium (Parizot et al., 2010). The inactivation of earlier active genes further contributes to the irreversibility of transitions and may also prevent the formation of LRs nearby already forming.

INCOMING CONNECTIONS

Soil is a highly heterogeneous and dynamic environment (Weil and Brady, 2016), with different nutrients, but also water and salt having highly distinct spatial patterns and temporal scales of change. To survive, plants must adapt their root system to these complex and variable conditions. To achieve this, plants can adapt the number, size, positioning, and angle of LRs in response to environmental conditions (Malamy, 2005; Gruber et al., 2013; Kellermeier et al., 2014). Here, we discuss the impact of a subset of environmental factors on lateral root development (Figure 4).

Water is of critical importance for plant survival, and its availability strongly affects plant root growth dynamics. Under conditions of drought, lack of water induces stress signaling that reduces root branching, while in case of patchy water availability, it acts as a positional cue for lateral root positioning (Robbins and Dinneny, 2015), with the absence of water having an additional abscisic acid (ABA)-dependent repressive effect (Orman-Ligeza et al., 2018). Finally, also LR growth angle is affected by water availability (Koevoets et al., 2016). Drought as well as salinity induces ABA signaling, which through its interplay with auxin, represses LRs (De Smet et al., 2003; Deak and Malamy, 2005; Duan et al., 2013; Promchuea et al., 2017) and to a much lesser extent main root elongation (De Smet et al., 2006). ABA signaling represses both later-stage LR outgrowth (De Smet et al., 2003), as well as LR initiation (Gibbs and Coates, 2014). The latter occurs via the *MYB93* transcription factor that is expressed in endodermal cells overlaying LR founder cells (Gibbs et al., 2014) and represses lateral root initiation. *MYB93* (*MYELOBLASTOSIS*) is auxin induced, ensuring that LR initiation only occurs in response to substantially strong and persistent elevations in auxin relative to surrounding auxin levels (Gibbs and Coates, 2014). Salt- or drought-induced ABA-dependent upregulation of *MYB93* elevates the threshold auxin levels required for LR initiation, thereby reducing LR formation (Figure 4, green boxes). In addition to ABA, salt-induced upregulation of the small signaling peptide *C-TERMINALLY ENCODED PEPTIDE 3* (*CEP3*) contributes to reduced LR growth (Delay et al., 2013). Hydropatterning, the biasing of LR formation in the direction of water availability, appears to be a conserved process observed in a variety of plant species, such as *Arabidopsis*, maize, and rice (Bao et al., 2014). Although the precise mechanism is currently still poorly understood, a clear role for auxin was identified while ABA signaling was shown not to be involved (Bao et al., 2014). Local auxin production through *TRYPTOPHAN AMINO-TRANSFERASE OF ARABIDOPSIS 1* (*TAA1*) and *PIN*-mediated transport were shown to contribute to increases in auxin levels and responses induced by water availability (Figure 4, blue boxes) (Bao et al., 2014) and result in the preferential induction of endodermal *PIN3* on the side exposed to water. Consistent with the role of this endodermal *PIN3* in LR initiation discussed earlier, hydropatterning appears to act at the earliest stages of LR formation: establishment of founder cell identity (Bao et al., 2014). The authors suggest that the upstream sensing of differences in water availability occurs close to the root tip and subsequently is somehow memorized (Bao et al., 2014).

Although at first perhaps counterintuitive, light has a major effect on plant root development (Mo et al., 2015; Lee et al., 2017; van Gelderen et al., 2018) (Figure 4, yellow boxes). Roots have been shown to predominantly display negative phototropism (Kutschera and Briggs, 2012; Wan et al., 2012), with mild

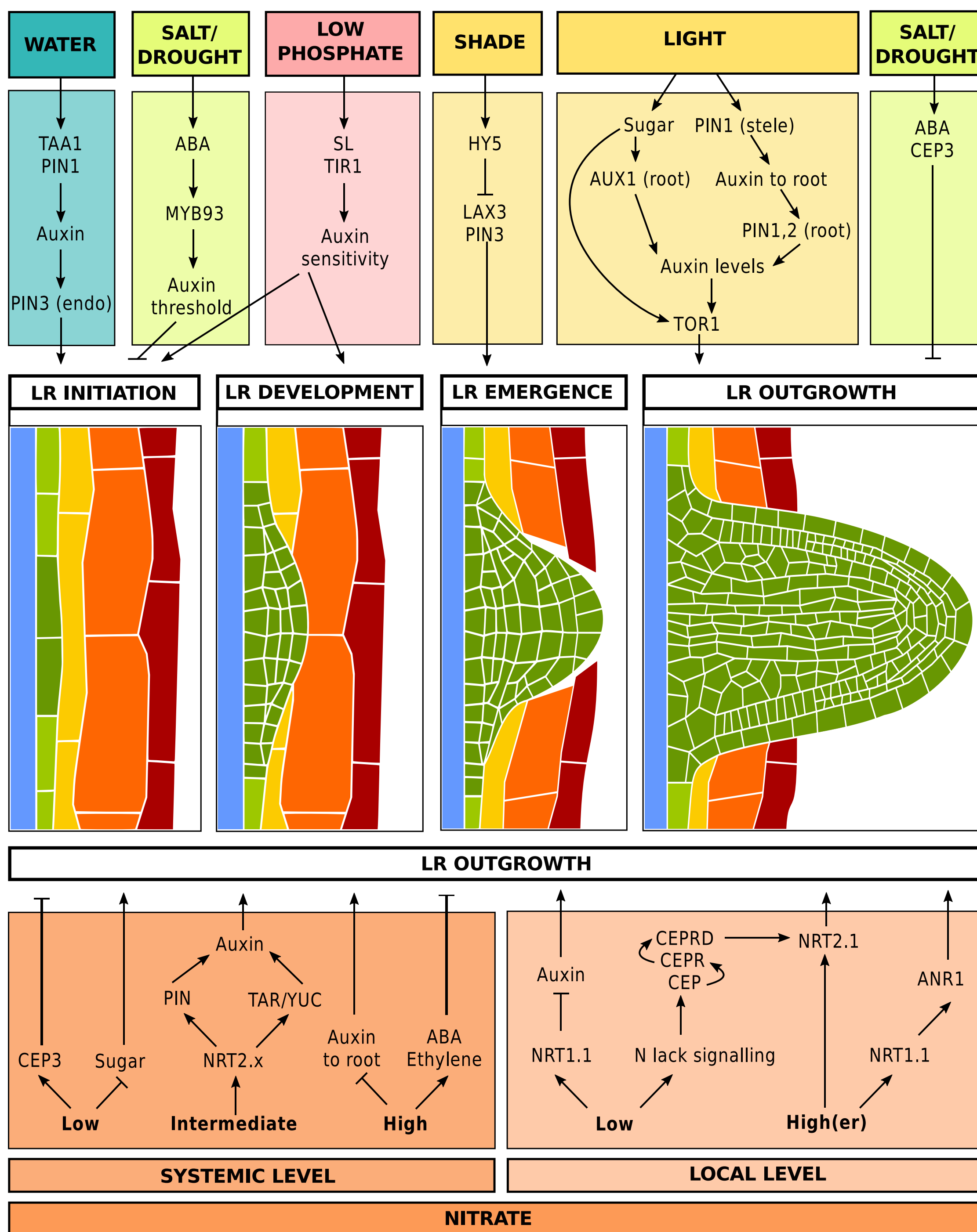

Figure 4. Overview of the Impact of a Subset of Environmental Factors on Lateral Root Development.
Schematic depiction of the impact of water, salt, drought, light, nitrate, and phosphate on lateral root development. For nitrate, the effects of both systemic nitrate levels, local nitrate levels, and signaling of nitrate lack are incorporated, which all affect lateral roots at the outgrowth stage. In contrast, water presence and phosphate deprivation affect laterals at the earliest stages of initiation and shade influences predominantly emergence, while salt and drought have effects at both early and late stages.

positive phototropic responses occurring to red light in the absence of gravity (Kiss et al., 2001, 2003; Ruppel et al., 2001). In addition, uniform light induces ROS signaling and affects root *PIN* patterns (Laxmi et al., 2008) and is likely to reflect a stress response (Yokawa et al., 2014). In addition to these direct effects of light on roots, also shoot light conditions have an impact on plant root architecture (van Gelderen et al., 2018). First, shoot light conditions affect photosynthesis, thereby affecting sugar production and hence shoot to root sugar transport (Kircher and Schopfer, 2012). In addition, shoot light conditions influence shoot to root auxin transport via *CONSTITUTIVE PHOTOMORPHOGENESIS1* (*COP1*)-dependent

modulation of plant stem *PIN1* levels, resulting in differential regulation of root *PIN1* and *PIN2* membrane levels (Sassi et al., 2012). Improved light conditions enhance both sugar and auxin delivery to the root (Reed et al., 1998; Bhalerao et al., 2002; Kircher and Schopfer, 2012), thereby enhancing root growth. Interestingly, sugars affect both auxin transport and biosynthesis (Mishra et al., 2009; Sairanen et al., 2012; Raya-González et al., 2017), and sugars and auxin converge on the same target of rapamycin (*TOR*) centered regulatory machinery for cell cycle and growth control (Henriques et al., 2014), enabling a highly coordinated control. Besides sugars and auxin, the light-dependent transcription factor, *ELONGATED HYPOCOTYL5* (*HY5*), was identified as influencing root development in response to changes in shoot light conditions (Oyama et al., 1997; Sibout et al., 2006). Shoot *HY5* production increases under a shading-induced shift to far red light, and *HY5* subsequently travels to the roots through the phloem (Chen et al., 2016). In addition, light itself has been suggested to be transported from shoots to roots (Sun et al., 2003, 2005), a phenomenon called stem-piped light transmission, thereby activating phytochrome receptors in the root (Lee et al., 2016), and thus potentially enabling local *HY5* induction. The relative importance of this mechanism remains unclear, particularly given the substantial reduction occurring in light transmission with increasing distance. *HY5* enhances its own expression (Abbas et al., 2014; Binkert et al., 2014), and this autoactivation may contribute to local, root-specific signal amplification. HY5 was recently shown to repress the auxin transporters, *LAX3* and *PIN3* (van Gelderen et al., 2018), explaining the observed inhibition of LR emergence from the repression of the *LAX3*/*PIN3*-mediated auxin accumulation in overlaying tissue layers (Péret et al., 2013).

Nitrogen (N) is the most limiting macronutrient for plant growth, with nitrate (NO^{-}_{3}) being the major inorganic source of this nutrient (Ruiz Herrera et al., 2015). Systemic levels as well as soil availability and soil distribution patterns have been shown to substantially influence root architecture (Figure 4, orange boxes) (Sun et al., 2017). In *Arabidopsis thaliana*, local nitrate availability is sensed close to the tip of primary roots and LRs. Under homogeneous nitrate conditions, root development is suppressed both under conditions of very low and very high nitrogen availability. Severe nitrogen deficiency has been shown to upregulate the LR-repressing, small signaling peptides *CEP1*, *3*, *4*, and *7* (Delay et al., 2013), with additional repression likely resulting from reduced sugar supply downstream of decreased photosynthetic activity. Also, in the case of nitrogen excess, systemic signals repress LR growth. Under these conditions, LR development is repressed by reducing auxin flow to the roots (Tian et al., 2008), induction of ethylene (Tian et al., 2009), and ABA signaling (Vidal et al., 2010), known to affect auxin levels and signaling, and modulation of the *AFB3*-miR393 pathway (Gifford et al., 2008; Vidal et al., 2014). Systemic repression likely also involves the glutamate receptors such as *GLR3.2* and *AtGLR3.4* which are phloem localized and repress LR initiation (Vincill et al., 2013). In contrast, for intermediate systemic nitrogen levels, LR development is promoted in a nitrate-dependent manner through *TAR* and *YUCCA*-mediated auxin biosynthesis and upregulation of *PIN*s occurring downstream of *NITRATE TRANSPORTER 2* (*NRT2*) nitrate transporters (Ma et al., 2014; Yu et al., 2014; Huang et al., 2015). In addition, nitric oxide, generated from nitrate, has been shown to promote LR formation (Sun et al., 2015).

In the case of spatially heterogeneous nitrate supply, an enhancement of LR growth on the high nitrate side and concomitant repression of LR growth on the low nitrate side occurs (Zhang and Forde, 1998; Little et al., 2005). The dual-affinity nitrate transporter *NRT1.1* (Liu et al., 1999) was shown to play an important role in this asymmetric growth response (Remans et al., 2006), by acting both as a nitrate transporter and sensor (Ho et al., 2009). Under low local nitrate availability, *NRT1.1* was shown to act as an auxin importer, and due to its position in the LR cap, this promotes the efflux of auxin out of the LR tip, thereby repressing LR growth (Krouk et al., 2010). In the presence of sufficient nitrate, *NRT1.1* only transports nitrate, thereby avoiding this negative affect. In addition, *NRT1.1* under these conditions further promotes LR development via *ARABIDOPSIS NITRATE REGULATED 1* (*ANR1*) (Zhang and Forde, 1998; Remans et al., 2006). Given that both auxin and nitrate induce *NRT1.1* expression, a positive feedback loop arises robustly promoting LR development in the presence of nitrate (Guo et al., 2002; Muños et al., 2004). In addition, also systemic signaling plays an important role in the asymmetric growth response. Roots at the low nitrate side were shown to produce a *CEP* signaling peptide, which upon perception in the shoot leads to the production of a downstream signal (Tabata et al., 2014). Co-occurrence of this signal with local nitrate perception on the other, high nitrate side of the root enhances *NRT2.1* expression (Ohkubo et al., 2017), thereby enabling an enhanced promotion of LR development.

Phosphate is another example of a nutrient whose soil availability is a major effector of plant root development (Williamson et al., 2001; Péret et al., 2011, 2014). Importantly, soil phosphate diffusion and leaching are considerably slower than that of nitrate, causing phosphate and nitrate to have contrasting, shallow versus deep soil distribution patterns under limiting conditions (Tinker and Nye, 2000). Under homogeneous low phosphate conditions, LR development is enhanced (Figure 4, pink boxes) while primary root growth is reduced. Both LR density and elongation are stimulated (Williamson et al., 2001; López-Bucio et al., 2002; Reymond et al., 2006; Jiang et al., 2007; Pérez-Torres et al., 2008). These morphological alterations of root system architecture enable so-called top soil foraging, the exploration of the superficial soil layers where phosphate is most likely to be found. The processes involved in the arrest of primary root growth were recently reviewed in detail elsewhere (Gutiérrez-Alanís et al., 2018). Stimulation of LR development involves phosphate starvation-induced upregulation of the auxin receptor *TIR1*, resulting in an enhanced auxin sensitivity that promotes primed pericycle cells to undergo LR initiation (Pérez-Torres et al., 2008). This *TIR1* upregulation was shown to be strigolactone dependent (Mayzlish-Gati et al., 2012). Under phosphate starvation, strigolactone was additionally demonstrated to reduce *PIN2* membrane levels (Kumar et al., 2015), thereby likely reducing LR tip auxin efflux and hence enhancing later stages of LR development. Besides responses to overall phosphate limitation, root architecture is also sensitive to phosphate distribution patterns, with local high phosphate levels inducing yet low levels repressing LR elongation (Drew, 1975; Linkohr

et al., 2002). Similar to the response to heterogeneous nitrate, this differential response involves communication between different plant parts (Liu et al., 1998; Burleigh and Harrison, 1999; Franco-Zorrilla et al., 2005), yet the precise nature of the long-distance signaling system and its integration with local phosphate sensing has so far remained elusive. In addition to water, gravitropic stimuli have also been found to influence the side of the root at which LR formation occurs (Lucas et al., 2008). Both the gravitropism-induced tissue curvature and downstream effects of cell shape changes on auxin accumulation (Laskowski et al., 2008) and mechanical Ca^{2+} dependent signaling have been implicated in this effect (Richter et al., 2009). Water availability and gravitropic stimuli likely act independently in biasing the sidedness of LR initiation, since root curvature appears to affect sidedness only after LRFC specification (Bao et al., 2014).

Plant roots need to simultaneously adjust to multiple and continuously changing environmental conditions. For this, integration between the signaling pathways set in motion by different environmental factors is of critical importance. As can be seen in Figure 4, for LR initiation multiple factors affect auxin levels, sensitivity or transport, enabling coordinated, weighted decision making on how often LRFCs should move on to LR initiation. Also, at later stages of LR development, environmental factors frequently target auxin processes, thereby enabling their integration. Besides converging on auxin, many more types of signal integration occur. As an example, we discussed in the above how both nitrate starvation and salt induce the signaling peptide *CEP3*, which represses LR outgrowth, enabling these factors to have additive repressive effects. Similarly, high systemic nitrate and salt or drought both induce ABA signaling. In contrast, light and low systemic nitrate levels have opposing effects on the delivery of sugar to the root.

In addition to different environmental factors converging on a shared signal, environmental factors may also have an impact on the plants response to other environmental factors. As an example, shoot light conditions, via the aforementioned *HY5*, have been shown to induce the nitrate transporter *NRT2.1* yet repress *NRT1.1* (Chen et al., 2016), thereby ensuring coordination of enhanced photosynthesis with enhanced nitrate uptake (Gangappa and Botto, 2016). Furthermore, light, by affecting the photosynthetic state and hence stomata opening and transpiration, has a direct effect on plant water dynamics. In addition to this direct link, circadian clock genes have been shown to control aquaporin expression (Takase et al., 2011) as well as auxin signaling (Voß et al., 2015). Shoot light conditions are thus likely to affect the threshold turgor and auxin levels required for LR emergence to occur and hence the response to other environmental factors.

We expect that many more signal convergence points will be discovered, and systems biology approaches will be essential to unravel how plants compute which decisions to reach based on their incoming information.

LOOSE ENDS

Particularly for founder cell identity establishment and lateral root initiation, many factors additional to the ones we have discussed have been found to be involved. Examples are *ABERRANT LATERAL ROOT FORMATION4* (*ALF4*), *MYB93*, Aurora kinases, *ARABIDOPSIS CRINKLY4* (*ACR4*), *GOLVEN6* (*GLV6*), *RAPID ALKALINIZATION FACTOR34* (*RALF34*), *CEP5*, *MEMBRANE-ASSOCIATED KINASE REGULATOR4* (*MAKR4*) (DiDonato et al., 2004; De Smet et al., 2008; Van Damme et al., 2011; Gibbs et al., 2014; Fernandez et al., 2015; Xuan et al., 2015; Murphy et al., 2016; Roberts et al., 2016). Many of these factors are involved in controlling the number, location, and orientation of the cell divisions initiating LRs and have an effect on lateral root spacing. *ALF4*, the expression of which is auxin independent, is required for maintaining pericycle cells in a mitosis competent state (DiDonato et al., 2004; Dubrovsky et al., 2008). Aurora kinases were found to play a critical role in the orientation of formative cell divisions, with mutants showing aberrant division plane orientations (Van Damme et al., 2011). *ACR4*, *GLV6*, *RALF34*, and *CEP5* are all involved in controlling the location and number of cell divisions (De Smet et al., 2008; Fernandez et al., 2015; Murphy et al., 2016; Roberts et al., 2016). This constriction of cell divisions to a limited region is essential for generating a dome-shaped LRP capable of normal emergence (Fernandez et al., 2015), as well as preventing the formation of nearby clustered LRP (De Smet et al., 2008; Murphy et al., 2016; Roberts et al., 2016). While *ACR4*, *GLV6*, and *RALF34* are all auxin inducible and division numbers and primordia clustering occurs in mutants, *CEP5* appears to be repressed by auxin, and its overexpression induces supernumerary division and LRP clusters. While we currently lack the data to understand how mitotic competence, location of cell cycle activation, and cell division orientation are properly integrated, it is apparent from the nature of the factors involved (small peptides, receptors) that these processes rely heavily on cell-cell signaling. For later stages of LR development, additional factors have been reported. As an example, the non-canonical auxin response factor ETTIN, as well as the transient closure of plasmodesmata, has been demonstrated to play a key role in LR emergence (Maule et al., 2013; Simonini et al., 2016).

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

In the current review, we provided an overview of what is currently known and can be hypothesized on LR development from a systems biology perspective. Specifically, we focused on how we can order distinct events within individual stages as well as connect the dots between the different stages of LR development. In addition, we considered how environmental conditions impinge on LR development and how computational models have helped increase our understanding of these processes.

An overwhelming number of molecular players involved in LR development have been identified, and likely many more are to be added. Here, we focused on those for which clear links have been established, and hence can be subjected to a systems biology analysis in terms of the motifs present in the regulatory networks and the temporal and spatial patterning dynamics these enable us to explain. One observation we made is that in an attempt to graphically summarize known data into regulatory network graphs, sometimes dangerous simplifications are made that can easily lead to misunderstandings. As an example,

often CWR enzymes are depicted as being regulated by *LAX3* (Taylor-Teeples et al., 2016; Du and Scheres, 2018). While the CWR enzymes are downstream of *LAX3* in the sense that they depend on *LAX3* activity for their induction, the fact that CWR enzymes are genetically regulated by the same factors as *LAX3* suggest that they simply require higher auxin levels, achieved through auxin import by *LAX3*. We thus recommend that care should be taken when drawing these summarizing regulatory graphs.

Our review of the data suggests that, for the sequence of transitions from priming to primordium patterning, positive feedback loops as well as repression of genes involved in earlier stages can be identified that may enable the robust transduction of one developmental stage into the next. Furthermore, we discuss how the subsequent transition to LR emergence can be envisioned as a propagating wave of auxin, with auxin inducing its own export and import into subsequent tissue layers. Following this propagating auxin wave, auxin-dependent targeted changes in cell wall properties and tissue turgidity subsequently enable LR emergence. To investigate these hypotheses, two complementary approaches are needed. First, substantial technical challenges need to be overcome to develop experimental setups capable of perturbing with spatiotemporal precision the positive feedbacks and repressive interactions identified, as well as within tissue auxin transport, water flows, and CWR. Furthermore, thus far, models have mostly focused on a single specific stage of LR development. Thus, a second important step is to extend models such that the temporal dynamics of developing from one LR stage into the next can be simulated, possibly first by simply morphing on LR shape into the next (Von Wangenheim et al., 2016).

However, apart from only modeling a single stage, current LR development models often also only consider a single process. As an example, for LR emergence and primordium development, the patterning of auxin in overlaying tissue layers, the auxin-dependent aquaporin dynamics changing tissue turgidity, and the cellular division patterns giving rise to a properly shaped layered primordium have thus far only been modeled in isolation (Péret et al., 2012, 2013; Von Wangenheim et al., 2016), while CWR has not yet been included in any model. It has therefore remained unclear how water transport, CWR, and tissue growth are coordinated, and to what extent their mutual interactions may synergistically contribute to the patterning and robustness of LR development. Indeed, models aimed at elucidating shoot meristem development and phyllotaxis that integrated gene regulation, hormone transport, and tissue mechanics have revealed important synergies between these processes (see e.g. Hamant et al., 2008; Heisler et al., 2010; Armezzani et al., 2018) Thus, new models eventually should combine core gene regulatory networks, hormone metabolism, and hormone transport with cellular growth and division, tissue mechanics, and water transport. Integrating these different processes within a model and investigating whether the incorporated interactions are necessary and sufficient to reproduce the available data (see e.g. Scheunemann et al., 2018) will be key to investigating how these processes together generate the specific spatiotemporal patterning of LRs in a robust and highly self-organized manner.

A well-known quote from the famous physicist Richard Feynman states "I do not understand what I can not build". In the context of LRs, this implies that obtaining an integrated systems-level understanding of LR development requires building more comprehensive models incorporating the different stages and processes. Such a model will be a valuable asset in our attempts to understand how roots integrate the multitude of environmental signals into their developmental programs such that they adapt themselves flexibly to their current situation. This ambitious aim will require the efforts of many experimentalists, bioinformaticians, and modelers alike.

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AUTHOR CONTRIBUTIONS

K.T.T. conceived the idea for the review, J.A.S.T. and K.T.T. wrote the manuscript. K.T.T. and J.A.S.T. conceived the figures. J.A.S.T. produced the figures.

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