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REVIEW

Lateral root formation and the multiple roles of auxin

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Abstract

Root systems can display variable architectures that contribute to survival strategies of plants. The model plant *Arabidopsis thaliana* possesses a tap root system, in which the primary root and lateral roots (LRs) are major architectural determinants. The phytohormone auxin fulfils multiple roles throughout LR development. In this review, we summarize recent advances in our understanding of four aspects of LR formation: (i) LR positioning, which determines the spatial distribution of lateral root primordia (LRP) and LRs along primary roots; (ii) LR initiation, encompassing the activation of nuclear migration in specified lateral root founder cells (LRFCs) up to the first asymmetric cell division; (iii) LR outgrowth, the 'primordium-intrinsic' patterning of *de novo* organ tissues and a meristem; and (iv) LR emergence, an interaction between LRP and overlaying tissues to allow passage through cell layers. We discuss how auxin signaling, embedded in a changing developmental context, plays important roles in all four phases. In addition, we discuss how rapid progress in gene network identification and analysis, modeling, and four-dimensional imaging techniques have led to an increasingly detailed understanding of the dynamic regulatory networks that control LR development.

Keywords: *Arabidopsis*, auxin, emergence, founder cell specification, initiation, lateral root, oscillation, outgrowth, primordium.

Introduction

Higher vascular land plants have successfully colonized the terrestrial environment via the evolution of multicellular organs that penetrate substrates, anchor plants, and absorb nutrients necessary for plant growth, termed root systems (Raven and Edwards, 2001; Pires and Dolan, 2012). As the main interface between plants and soil, root systems adapt to very heterogeneous macro- and micro-conditions, such as soil composition, subterranean competition with other plants, and rhizosphere abiotic- and biotic-interactions (López-Bucio *et al.*, 2003; Hodge, 2006; Philippot *et al.*, 2013). Therefore, diversity and plasticity in root architecture may significantly contribute to survival strategies of plants.

Root systems consist of roots derived from the embryo (embryonic roots) and roots derived from existing roots or non-root tissues (post-embryonic roots) (Atkinson *et al.*, 2014). Post-embryonic roots arising from existing roots are termed lateral roots (LRs), while roots arising from non-root tissues are termed adventitious roots (Atkinson *et al.*, 2014). In dicots, the embryonic root commonly becomes dominant, forming a thick central taproot, from which secondary LRs usually develop (Bellini *et al.*, 2014). LRs then reiterate LR formation and develop the higher-ordered and spatially separated root system, termed the taproot system (allorhizic root system) (Osmont *et al.*, 2007; Bellini *et al.*, 2014). In taproot systems, embryonic roots are usually important during the entire life cycle of the plant (Bellini *et al.*, 2014). In contrast to the taproot system, the majority of monocot roots form a fibrous 'homorhizic' root system, which is characterized by the development of many adventitious roots (Osmont *et al.*, 2007; Bellini *et al.*, 2014). Embryonic roots in this system are usually small, short-lived, and only important at early stages of seedling development (Bellini *et al.*, 2014).

In the taproot system, the growth of embryonic roots (also termed primary roots) and LRs are the major determinants of root system architecture. Here, we focus on LR formation in the dicotyledonous model plant *Arabidopsis thaliana*. In arabidopsis roots, layers of epidermal, cortical, and endodermal tissues surround a single-layered pericycle and central vasculature tissues (Figs 1 and 2A). The pericycle is a heterogeneous tissue with diarch symmetry composed of two cell types, sitting in front of xylem- and phloem-pole, respectively, with different cytological features and cell fates (Fig. 2A) (Beeckman *et al.*, 2001; Himanen *et al.*, 2004; Laplaze *et al.*, 2005; Mähönen *et al.*, 2006; Parizot *et al.*, 2008). Different from quiescent phloem-pole-pericycle (PPP) cells, xylem-pole-pericycle (XPP) cells are thought to be 'semi-meristematic', based on the characterization of their ultrastructure (small vacuoles, dense cytoplasm and ribosomes) and cell division ability (XPP cells remain in the G2 phase of the cell cycle for a longer period than PPP cells) (Beeckman *et al.*, 2001; Himanen *et al.*, 2004; Parizot *et al.*, 2008). In accordance with this, XPP cells are exclusively competent for LR formation. The specification of XPP cells for their 'semi-meristematic' potency innately correlates with vascular pattering of the xylem lineage (Parizot *et al.*, 2008).

LRs initiate acropetally, with different developmental stages (from young to old) positioned at different longitudinal positions along the primary root (from root tip to shoot) (Fig. 1) (Dubrovsky *et al.*, 2006, 2011). The growing root of arabidopsis can be divided into four developmental zones according to their cellular activities (Fig. 1) (Dolan *et al.*, 1993; Verbelen *et al.*, 2006). From root tip to shoot, these are: (i) the meristem, the zone with active cell divisions; (ii) the transition zone (TZ), also termed the basal meristem, where cell division competence is still present but is accompanied by slow cell growth in both length and width; (iii) the elongation zone (EZ), the zone with rapid and extensive cell elongation without growth in width; and (iv) the differentiation zone (DZ), the zone in which cells cease to expand and start to differentiate to their specialized features (Fig. 1) (Dolan *et al.*, 1993; Verbelen *et al.*, 2006). In the most-rootward part, the root cap – including the lateral root cap (LRC) and columella (COL) – is thought to form protective and sensory tissue layers to shield the meristematic cells during soil penetration and to perceive environmental signals (Figs 1 and 2C) (Barlow, 2002; Morita, 2010).

Chronologically, LR formation has been divided into four steps (Fig. 1), as follows. (i) LR positioning (mostly in TZ and EZ), which encompasses mechanisms to position, specify, and activate lateral root founder cells (LRFCs), thereby regulating the spatial distribution of lateral root primordia (LRP) and LRs along primary roots. (ii) LR initiation (mostly in early DZ), a phase encompassing the activation of nuclear migration in specified LRFCs up to the stereotypical first asymmetric cell division. (iii) LR outgrowth (in DZ), the 'primordium-intrinsic' establishment of new organ tissues and meristem in an orthogonal axis to the parental roots after the initiation. (iv) LR emergence (in DZ), an interactive process between LRP and their overlaying tissues to allow passage through cell layers and the birth of new LRs. Newly formed LRs possess *de novo* patterned root tissues and meristems greatly resembling those in the primary roots to ensure their continuous growth (Fig. 2C).

Lateral root positioning

Rhizotaxis, defined as the arrangement of lateral organs (emerged LRs) along plant roots (Laskowski *et al.*, 2008; Hofhuis *et al.*, 2013), is a joint outcome of LR formation (including all four steps). However, rhizotaxis is not sufficient to reflect the main mechanisms of positioning. Instead, preparatory steps for LR initiation, including the oscillation and LRFC specification processes described below, can be considered as the decisive positioning process for setting up the branching pattern of the root system, which encompasses to the spatial distribution of LRP/LRs. For arabidopsis roots grown under experimental conditions, the spatial distribution of LRP/LRs is measured as a combination of primary root growth (root length), LRP/LR density, and the minimum distance of two successive LRP/LRs (by making use of suitable markers to visualize LRFCs, LRP, and LRs) (Dubrovsky *et al.*, 2006; Hofhuis *et al.*, 2013).

Oscillation: an intrinsic mechanism to pre-pattern LR initiation sites

Molecular evidence suggests that early events to pattern LRs initially occur in the root tips. In the basal meristem, recurrent expression of the synthetic *DR5* (*DIRECT REPEAT5*) auxin-responsive promoter fused to the *β-glucuronidase*

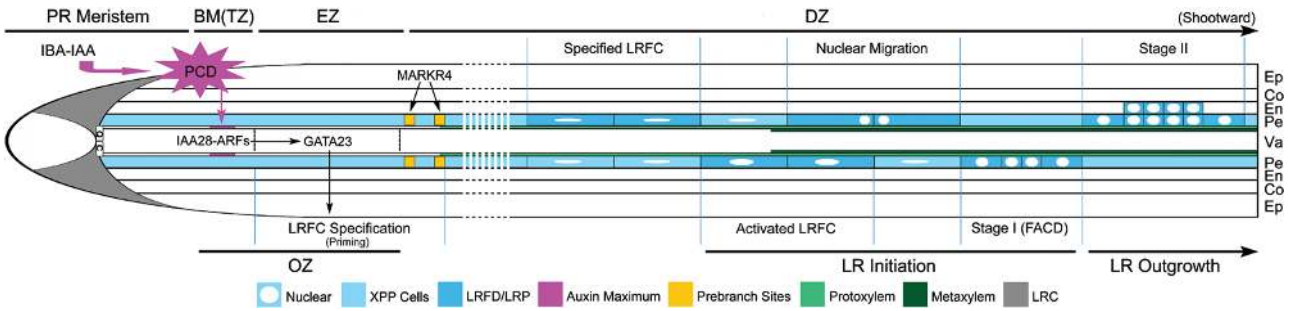

Fig. 1. LR positioning initiates in the basal meristem and progresses until XPP cells reach the differentiation zone. PR Meristem, primary root meristem; TZ, transition zone; BM, basal meristem; EZ, elongation zone; OZ, oscillation zone; DZ, differentiation zone; FACD, first asymmetric cell division; QC, quiescent center; LRC, lateral root cap; Ep, epidermis; Co, cortex; En, endodermis; Pe, pericycle; XPP, xylem pole pericycle; Va, vasculature; LRFC, lateral root founder cell; LRP, lateral root primordium; LR, lateral root; PCD, programmed cell death.

(*GUS*) reporter gene is observed in protoxylem cells at both sides with a period of 15 h (Fig. 1) (De Smet *et al.*, 2007). *DR5:GUS* expression sites in the basal meristem have been shown to correlate with subsequent sites of LRP by tracing toner ink-labels at the basal meristem over time (De Smet *et al.*, 2007). In line with this, fusions of the *DR5* promoter to the *luciferase* (*LUC*) reporter gene reveal an *in vivo* behavior of oscillatory activity of *DR5:LUC* in the root tip (Moreno-Risueno *et al.*, 2010). The dynamic *DR5* expression pattern occurs in a relatively broad region termed the oscillation zone (OZ), which covers the basal meristem and elongation zone (Fig. 1) (Moreno-Risueno *et al.*, 2010; Van Norman *et al.*, 2013). The cells involved in each peak of the *DR5* oscillation become a region with stable *DR5* expression in the early DZ, designated the 'LR pre-branch site', which is thought to mark regions with the competence to develop an LRP (Fig. 1) (Moreno-Risueno *et al.*, 2010; Van Norman *et al.*, 2013).

DR5 expression is commonly considered as a proxy of auxin distribution, but auxin itself has been argued not to be sufficient to specify the oscillatory *DR5* behavior in the OZ and the subsequent formation of LR pre-branch sites (Moreno-Risueno *et al.*, 2010; Van Norman *et al.*, 2013). This conclusion is based on the failure of exogenously applied auxin in the OZ to trigger new LR pre-branch sites and the observation that not all auxin-responsive genes exhibit periodic expression as the *DR5* reporter in the OZ (Moreno-Risueno *et al.*, 2010; Van Norman *et al.*, 2013). Analysis of gene expression in the OZ led to the conclusion that thousands of genes display an oscillation pattern that are in phase or in antiphase with the *DR5* reporter, suggesting that this oscillation reflects a large-scale developmental response (Moreno-Risueno *et al.*, 2010). Several candidate transcriptional regulators are confirmed to exhibit oscillatory expression and function in LR development, including AUXIN RESPONSIVE FACTOR7 (ARF7) and LATERAL ORGAN BOUNDARIES DOMAIN16 (LBD16) (Okushima *et al.*, 2007; Moreno-Risueno *et al.*, 2010). In particular, *arf7* mutants show aberrant oscillations and irregular pre-branch sites marked by *DR5:LUC*, suggesting that ARF7 is important for periodic gene expression in the OZ (Moreno-Risueno *et al.*, 2010). Taken together, periodic gene activities in the OZ, which at least partially correlate with auxin signaling, constitute an early step to position new LRP/LRs.

With regard to the oscillation model, there are still questions that remain to be elucidated. Due to the low spatial resolution of *DR5:LUC* expression, the precise spatial domain for oscillation has not yet been described. The cellular information provided by the oscillation is proposed as a priming step (Fig. 1), from which XPP cells should receive signals from the adjacent *DR5:GUS*-marked protoxylem cells during the oscillation (De Smet *et al.*, 2007). How this priming step is achieved is currently unclear. Moreover, the oscillating period visualized by *DR5:LUC* is 4 to 6 h (Moreno-Risueno *et al.*, 2010; Xuan *et al.*, 2015, 2016), which appears to be shorter than the frequency of successively initiated LRP/LRs and the repetitive period indicated by *DR5:GUS* (Dubrovsky *et al.*, 2006; De Smet *et al.*, 2007), suggesting that other regulatory mechanisms are required for converting cells with competence after the oscillation to 'true' LRP/LR initiation sites (Fig. 1) (Van Norman *et al.*, 2013).

Auxin derived from the lateral root cap modulates oscillation

An auxin source in lateral root cap (LRC) cells is able to influence the LRP/LR initiation sites (Casimiro *et al.*, 2001; Swarup *et al.*, 2005; De Smet *et al.*, 2007; Xuan *et al.*, 2015, 2016). This phenomenon was first discovered from tissue-specific complementation experiments in agravitropic *auxin resistant1* (*aux1*) mutant roots (Swarup *et al.*, 2005; De Smet *et al.*, 2007). The *AUX1* gene encodes an auxin influx carrier member that facilitates auxin transport (Bennett *et al.*, 1996). Roots in the *aux1* mutant bend constitutively in one direction, forming root coils with LRs predominantly distributed on the convex side of the curve, which markedly differs from the wavy pattern displayed in wild-type roots growing under the same conditions (Swarup *et al.*, 2005; De Smet *et al.*, 2007). In addition, reduced LR density and oscillatory activity in the basal meristem (marked by *DR5:GUS*) are observed in *aux1* mutant roots (Marchant *et al.*, 2002; De Smet *et al.*, 2007). Targeted expression of *AUX1* to LRC and epidermal tissues fully restores not only the *aux1* agravitropic phenotype but also the aberrant branching pattern (Swarup *et al.*, 2005; De Smet *et al.*, 2007).

A recent small-molecule screen from a compound library for LR initiation identified naxillin as a non-auxin molecule that stimulates the conversion of the auxin precursor indole-3-butyric acid (IBA) to the active auxin indole-3-acetic acid (IAA) in the root cap (De Rybel *et al.*, 2012). The local root cap IBA-to-IAA conversion efficiency positively modulates the oscillation amplitude, determined by the *DR5:LUC* intensity in the OZ, thereby stimulating the formation of LR pre-branch sites and subsequent LRP/LRs (Fig. 1) (De Rybel *et al.*, 2012; Xuan *et al.*, 2015). Tissue-specific analysis reveals that the IBA-to-IAA conversion pathway is active specifically in the outer LRC cells to produce IBA-derived auxin, which is assumed to be transported toward the OZ (Fig. 1) (Xuan *et al.*, 2015, 2016). This transport correlates with the release of auxin pulses from the most-distal LRC cells (in the OZ) during their recurrent programmed cell death (PCD) and the accumulation of auxin in abutting epidermal cells (Fig. 1) (Xuan *et al.*, 2016). And the periodicity of PCD-derived auxin correlates with the oscillation and the formation of LR pre-branch sites and subsequent LRP/LRs (Fig. 1) (Xuan *et al.*, 2016). However, how this PCD-derived auxin penetrates root tissues to drive the oscillation and thereby influences the LRP/LR initiation sites remains to be determined. Regardless of the precise mechanisms involved, the dynamics of root cap turnover may co-ordinate primary root growth with root branching in response to environmental stimuli, such as gravity, water, and nutrients (Xuan *et al.*, 2016). These findings highlight possible interaction sites in plants to transform extrinsic environmental signals into intrinsic developmental strategies.

Lateral root founder cell specification

Lateral root organogenesis commences with the specification of LRFCs, a process to select a subset of competent XPP cells to initiate LRP (Fig. 1). Oscillation might be necessary but not sufficient for LRFC specification. During oscillation, a group of pericycle cells may exit from the OZ, from which only pairs of abutted pericycle cells are specified as LRFCs (Van Norman *et al.*, 2013). This implies that mechanisms may exist to locally refine or restrict the number of pericycle cells to become specified LRFCs during and/or after the oscillation (Van Norman *et al.*, 2013). LR pre-branch sites are created with static *DR5:LUC* expression in the early DZ after the oscillation (Moreno-Risueno *et al.*, 2010). However, not all 'static' points of *DR5* expression generated from the oscillation persist to become LR pre-branch sites and then LRP/LRs, which has been observed under strong gravistimulation (Moreno-Risueno *et al.*, 2010). Therefore, it might be reasonable to speculate that LR pre-branch sites might reflect activated LRFCs, and 'non-persistent' static points of *DR5* expression might reflect competent XPP cells that are not activated. In this scenario, LRFCs are assumed to be specified before entering into the DZ, in other words, during the oscillation. Alternatively, keeping the limited cellular resolution of *DR5:LUC* in mind, LR pre-branch sites might also indicate a broader, competent site from which LRFC specification is initiated subsequently (Van Norman *et al.*, 2013). In this scenario, LRFC specification happens after and separately from the oscillation.

The auxin-regulatory GATA23 transcription factor is considered as the first molecular marker for LRFCs (De Rybel *et al.*, 2010). An auxin signaling cascade involving INDOLE-3-ACETIC ACID28 (IAA28) and its interacting ARF binding factors (ARF5, 6, 7, 8, and 19) in the basal meristem controls *GATA23* expression in XPP cells that leave the basal meristem (Fig. 1) (De Rybel *et al.*, 2010). The *GATA23* expression pattern presents broad patches with elevated peaks that can mark LRFCs and LRP at early stages (De Rybel *et al.*, 2010). Both *GATA23* loss- and gain-of-function mutants display altered numbers and spacing of LRP and LRs, suggesting a role in specifying LRFCs (De Rybel *et al.*, 2010). However, it is not clear whether GATA23 regulates LRFC specification during/through the oscillation or after the oscillation. Another candidate regulator of LRFC specification is MEMBRANE-ASSOCIATED KINASE REGULATOR4 (MARKR4) that has been identified as a downstream molecular component of the IBA-to-IAA conversion pathway (Xuan *et al.*, 2015). MARKR4 is required to convert a LR pre-branch site into LRP after the oscillation as *markr4* loss-of-function mutant roots show fewer LRP/LRs but unaltered LR pre-branch sites (Fig. 1) (Xuan *et al.*, 2015). In addition, AtMYB93, which belongs to the subfamily of R2R3 MYB (MYELOBLASTOSIS) transcription factors, can be specifically induced in the basal meristem by auxin, hinting at its potential role in oscillation and/or LRFC specification (Gibbs *et al.*, 2014).

The bilateral periodic pre-patterning spanning both xylem poles (visualized by *DR5:GUS*) is usually followed by unilateral initiation of LRP from one xylem pole. Therefore, LRFC specification must encompass mechanisms to determine the sidedness of the initiation. Curvature has been considered as a decisive cue to specify the sidedness of LRFCs (Kircher and Schopfer, 2016). Despite an independence between LR initiation and gravity (Ditengou *et al.*, 2008; Lucas *et al.*, 2008; Richter *et al.*, 2009; Moreno-Risueno *et al.*, 2010; Paul *et al.*, 2012; Van Norman *et al.*, 2013), both gravity and mechanical force-induced bends influence LR initiation sites, most of which reside on the convex side of a curve (De Smet *et al.*, 2007; Ditengou *et al.*, 2008; Laskowski *et al.*, 2008; Lucas *et al.*, 2008; Richter *et al.*, 2009; Kircher and Schopfer, 2016). An *in silico* root system indicated that the differential XPP cell geometry between two sides of the curve, in which cells are slightly stretched on the convex side and compressed on the concave side, might act as a trigger to determine the sidedness of LRFCs (Laskowski *et al.*, 2008). The stretched XPP cells are more competent than other cells to form a robust new auxin maximum (Laskowski *et al.*, 2008). A *D-type cyclin cycd4;1* mutant shows an increased pericycle cell length in the OZ and reduced LR density, hinting at a role of cell geometry in LRFC specification and/or oscillation (Nieuwland *et al.*, 2009). However, other research has challenged this hypothesis by performing a transient bending assay, in which no obvious asymmetric geometry of the XPP cells was observed after repositioning the root from a 20-s period of mechanical bending (Richter *et al.*, 2009). This transient bending is, however, sufficient to position LRs on the convex side of formerly bent regions. A rapid heterogeneous change of cytosolic Ca^{2+} levels upon stretching has been proposed to determine the sidedness and drive the LRFC specification (Richter *et al.*, 2009). Independent from curvature, a non-uniform distribution of water availability influences the distribution of LRP/LRs and might act at the time of LRFC specification to determine the sidedness after the oscillation (Bao *et al.*, 2014).

Auxin and auxin signaling participate in regulating LRFC specification. A heat-shock inducible system was used to create clonal sectors randomly in roots, including patches in XPP cells, in which an auxin biosynthetic enzyme *indoleacetic acid tryptophan monooxygenase* (*iaaM*) is expressed to increase auxin production (Dubrovsky *et al.*, 2008). Additional LRP were initiated from auxin-producing pericycle sectors, indicating that a local auxin input is able to specify LRFCs, perhaps regardless of the tissue context along the primary root (Dubrovsky *et al.*, 2008). From the *in silico* root system referred to above, a lateral inhibition theory has been proposed, in which the 'existing' specified and/or specifying LRFCs will inhibit new founder cells from being specified adjacently in the distal (rootward) region (Laskowski *et al.*, 2008). This requires proper expression of auxin influx and efflux carriers, which may contribute to the formation of a robust new auxin maximum in selected XPP cells and the depletion of the auxin level in their surrounding cells (Laskowski *et al.*, 2008). Consistently, mutants in auxin polar transport and auxin signaling show altered branching pattern, with closely grouped (or even fused) LRP/LRs or fewer LRP/LRs, such as *pin2pin3pin7*, weak alleles of *gnom*, *shy2* (*short hypocotyl2/iaa3*; gain-of-function), *bdl* (*bodenlos/iaa12*;

gain-of-function), and *mp* (*monopteros*/*arf5*) mutant roots (Geldner *et al.*, 2004; Laskowski *et al.*, 2008; De Smet *et al.*, 2010; Goh *et al.*, 2012*b*; Okumura *et al.*, 2013). Moreover, three PLETHORA (PLT) transcription factors PLT3, PLT5, and PLT7, act downstream of the ARF7/ARF19-mediated signaling pathway to prevent the formation of primordia close to one another (Hofhuis *et al.*, 2013). In *plt3plt5plt7* loss-of-function mutant roots, successive LRP are frequently grouped in clusters (Hofhuis *et al.*, 2013). Recently, the auxin-responsive small signaling peptides C-TERMINALLY ENCODED PEPTIDE 5 (CEP5) and RALFL34 have been shown to affect root architecture, because LRP/LRs are unusually positioned (sometimes clustered) in their gain/loss-of-function mutant roots (Murphy *et al.*, 2016; Roberts *et al.*, 2016).

Ectopic over-production of cytokinin in the zone encompassing the basal meristem by tissue-specific activation of the cytokinin biosynthesis gene *ISOPENTENYL TRANSFERASE* (*IPT*) results in fewer initiated LRP/LRs (Bielach *et al.*, 2012). Therefore, in addition to auxin, enhanced cytokinin responses in pericycle cells between existing LRP might restrict other primordia to 'ectopically' initiate near the existing LRP, thereby preventing 'extra' LRFCs from being specified after the oscillation (Bielach *et al.*, 2012).

Mobile signals have been proposed to regulate LRP/LR positioning through interconnected signaling pathways (De Smet *et al.*, 2008; Notaguchi *et al.*, 2012; Benitez-Alfonso *et al.*, 2013; Vincill *et al.*, 2013). A dynamic symplastic transport is observed in XPP cells (including LRFCs) and LRP, where positive cell-to-cell connectivity between the pericycle cells and early-stage primordia becomes restricted in older LRP (Benitez-Alfonso *et al.*, 2013). By interrupting callose degradation around plasmodesmata (PD), *plasmodesmal-localized β-1,3-glucanase1* (*pdbg1*)*pdbg2* double-mutant roots are impaired in symplastic connectivity and exhibit an altered branching pattern, in which LRP/LRs are closely grouped or fused (Benitez-Alfonso *et al.*, 2013). *PdBG1* is expressed at low levels in the vasculature (including the basal meristem), LRFCs, and incipient LRP; while *PdBG2* is expressed in the stele and LRP (Benitez-Alfonso *et al.*, 2013). Based on the expression domain of *PdBG1* and *PdBG2* genes, they may control LR positioning by influencing the transport of inhibitory mobile signals, probably from existing LRFCs and/or LRP to the neighboring tissues (Benitez-Alfonso *et al.*, 2013). Moreover, receptor-like proteins and kinases involved in cell-to-cell communication are reported to control the spatial distribution of LRP/LRs (De Smet *et al.*, 2008; Vincill *et al.*, 2013). GLUTAMATE RECEPTOR-LIKE3.2 (GLR3.2) and GLR3.4 proteins function as amino acid-gated Ca^{2+} channels at the plasma membrane. *GLR3.2* and *GLR3.4* are transcribed in the phloem, where they are able to regulate LR positioning (perhaps through the oscillation) by transporting mobile signals (Vincill *et al.*, 2013). Another membrane-localized receptor-like kinase ARABIDOPSIS CRINKLY4 (ACR4), which resides specifically in the small daughter cells after the first asymmetric cell division of LRFCs, prevents neighboring pericycle cells from being specified into LRFCs non-cell autonomously (De Smet *et al.*, 2008).

The evidence described above shows that channels for gating interconnective signals are important for LR positioning, while evidence for mobile signals is missing. The phloem-mobile *IAA18* mRNA is the first reported mobile signal that is able to travel from the vascular tissue of mature roots and leaves to the basal meristem, where the *IAA18* gene is not transcribed, and might be functional in the oscillation and/or LRFC specification (Notaguchi *et al.*, 2012). Another mobile signal that may potentially regulate LRP/LR spacing is a small peptide from the GOLVEN/ROOT GROWTH FACTOR/CLE-like (GLV/RGF/CLEL) family, GLV6 (Fernandez *et al.*, 2015). Ectopically expressed GLV6 in the LRP, endodermis, and epidermis is able to induce anticlinal and periclinal divisions in XPP cells (Fernandez *et al.*, 2015).

Lateral root initiation

Specified LRFCs are assumed to subsequently receive 'activating' signals to start nuclear migration toward the central common cell wall, during which the spindle-like nuclear shape starts to swell and is transformed during migration to the nearly round shape observed before the division (Figs 1 and 2B). Therefore, the start of nuclear shape transformation has been considered as a sign of LRFCs being activated and as an indicator for the start of LR initiation. LR initiation is defined as being finished when the first formative divisions are accomplished and the proper daughter cell fates are established (Figs 1 and 2B) (De Smet *et al.*, 2006). Another visible morphological change during LR initiation is the swelling of LRFCs concomitant with the nuclear migration (Fig. 2B) (Vermeer *et al.*, 2014). A stereotypical bi-cellular mode of LR initiation is frequently observed, in which the first anticlinal cell divisions occur asymmetrically in adjacent founder cells to generate two short daughter cells flanked by two longer cells (Figs 1 and 2B) (Casimiro *et al.*, 2001; Dubrovsky *et al.*, 2001; De Smet *et al.*, 2008; De Rybel *et al.*, 2010; Lucas *et al.*, 2013; Fernandez *et al.*, 2015; von Wangenheim *et al.*, 2016). The first asymmetric cell division occurs within 4–7 h after the onset of nuclear repositioning (De Rybel *et al.*, 2010). A successful LR initiation demands tight interplay between cell division and cell fate respecification.

Signaling inputs for cell division

In specified LRFCs, an auxin maximum is created and maintained via activation of auxin biosynthesis and the auxin influx carrier AUX1 (Fig. 2B) (Laskowski *et al.*, 2008; Tang *et al.*, 2017). Plant-specific B3 transcription factors FUSCA3 (FUS3) and LEAFY COTYLEDON2 (LEC2) interact with each other to induce expression of the auxin biosynthetic gene *YUCCA4* in LRFCs (Tang *et al.*, 2017). Moreover, the expression of the synthetic auxin response *DR5* reporter precedes the first asymmetric cell division and starts along with the nuclear migration (De Rybel *et al.*, 2010), indicating that auxin signaling is associated with LR initiation. An important auxin signaling component for LR initiation is the SOLITARY-ROOT (SLR)/IAA14-ARF7/ARF19 module,

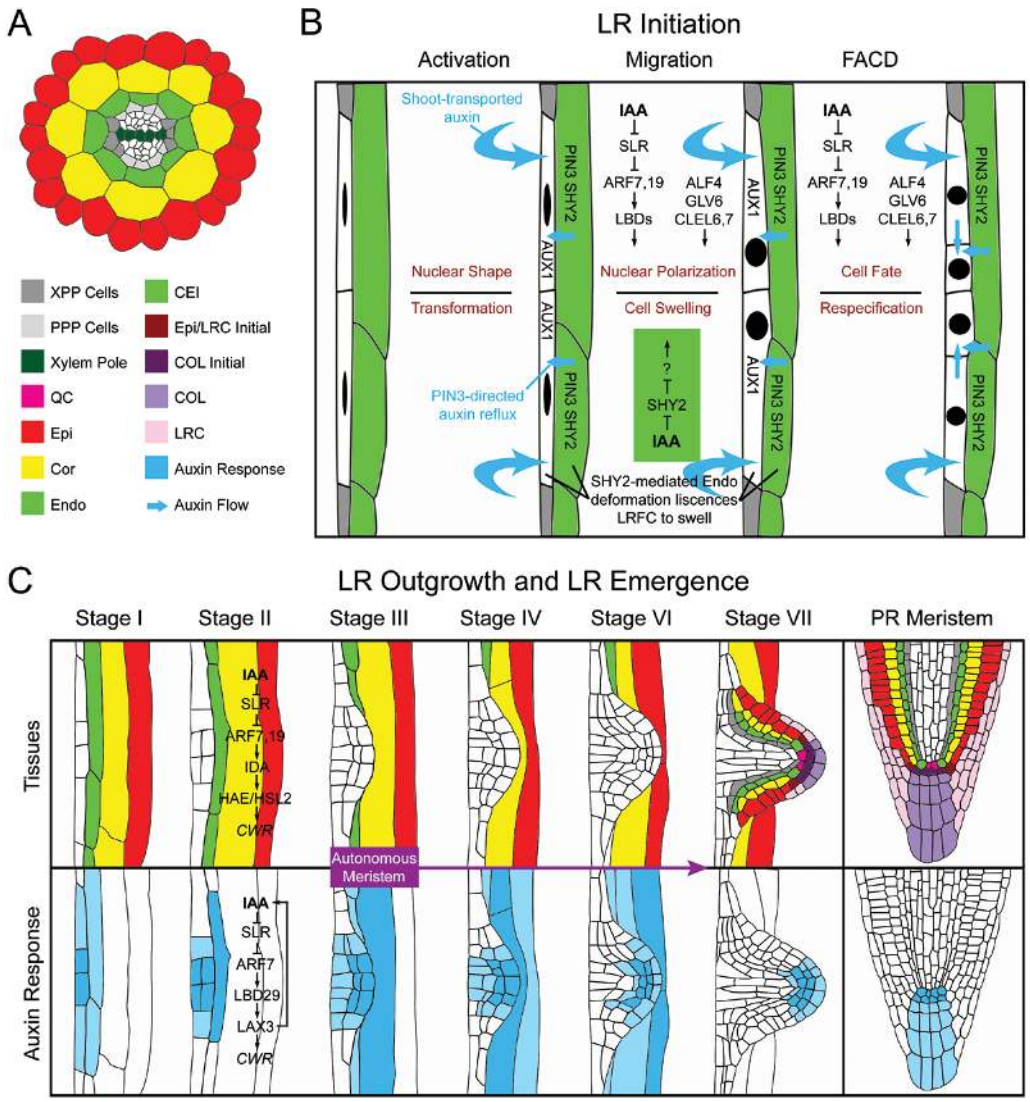

Fig. 2. LR initiation, outgrowth, and emergence commence with nuclear migration in lateral root founder cells and culminates in the emergence of a LR with an organized meristem. (A) Schematic representation of arabidopsis primary root cross-section. (B) Main signaling events and auxin flow during LR initiation. (C) Main signaling events and auxin response during LR outgrowth and emergence. XPP: xylem pole pericycle; PPP: phloem pole pericycle; QC: quiescent center; CEI: cortex/endodermis initial; Endo: endodermis; Cor: cortex; Epi: epidermis; LRC: lateral root cap; COL: columella; PR: primary root; FACD: first asymmetric cell division; LRFC: lateral root founder cell; *CWR*: *Cell Wall Remodeling* genes.

in which auxin-induced degradation of labile SLR proteins de-represses ARF7 and ARF19 transcription factors to activate downstream gene expression (Fukaki *et al.*, 2002, 2005; Okushima *et al.*, 2005; Wilmoth *et al.*, 2005). LRP and LRs are virtually absent from both dominant negative *slr* and loss-of-function *arf7arf19* mutant roots (Fukaki *et al.*, 2002, 2005; Okushima *et al.*, 2005; Wilmoth *et al.*, 2005). The cell-cycle-associated B-type cyclin *CYCB1;1* reporter that marks the G2–M transition is strongly expressed in LRFCs undergoing the first divisions (Himanen *et al.*, 2002; Vanneste *et al.*, 2005). The lack of *CYCB1;1* expression in *slr* and *arf7arf19* mutant roots indicates a cell cycle block during LR initiation controlled by SLR and ARF7/ARF19 (Fig. 2B) (Vanneste *et al.*, 2005; Okushima *et al.*, 2007). In addition, LRP/LR density negatively correlates with SLR degradation rate (monitored by different point-mutated SLR protein variants), in which LRP/LRs density decreases in roots harboring slow-degraded SLR variants (Guseman *et al.*, 2015). This suggests that SLR is an auxin-initiated timer for LR initiation (Guseman *et al.*, 2015). Moreover, BRASSINOSTEROID-INSENSITIVE2 (BIN2)-mediated phosphorylation of ARF7 and ARF19 attenuates their interaction with IAAs, including SLR, and subsequently enhances the transcriptional activity to their target genes (Cho *et al.*, 2014). In line with this, gain-of-function *bin2* mutants show increased LRP/LR density (Cho *et al.*, 2014).

In addition to SLR-mediated auxin signaling in LRFCs, SHOOT HYPOCOTYL2 (SHY2)/IAA3-mediated auxin signaling in the overlaying endodermal cells has been reported to be crucial for LR initiation (Fig. 2B) (Vermeer *et al.*, 2014; Vermeer and Geldner, 2015). SHY2 degradation in the endodermis induces mechanical feedback through cell volume loss to accommodate the turgidity of the underlying LRFCs, thereby allowing subsequent cell divisions to initiate LRP (Fig. 2B) (Vermeer *et al.*, 2014). In line with this, the physical elimination of endodermal cells triggers XPP cells to swell and to re-enter

the cell cycle with the activation of *CYCB1;1* expression (Marhavý *et al.*, 2016). The auxin efflux carrier PIN3 is considered to be involved in these auxin-induced mechanical changes (Fig. 2B) (Marhavý *et al.*, 2013). PIN3 is transiently induced in the endodermal cells overlying LRFCs when nuclear migration takes place. PIN3 stimulates LR initiation, as LRFCs in *pin3* mutant roots are temporally delayed in accomplishing the first asymmetric cell divisions (Marhavý *et al.*, 2013). In the overlaying endodermal cells, PIN3 localizes toward the inner membrane adjacent to the LRFCs, presumably creating auxin reflux to the founder cells (Fig. 2B) (Marhavý *et al.*, 2013).

Direct downstream components of ARF7 and ARF19 have been identified, among which members of the LBD proteins have been shown to function in LR development, including LR initiation (Fig. 2B) (Okushima *et al.*, 2005, 2007; Berckmans *et al.*, 2011; Goh *et al.*, 2012*a*). LBD18 and LBD33 protein dimers mediate LR initiation by direct binding to the promoter of the *E2Fa* gene, which encodes a transcriptional activator of cell cycle genes (Berckmans *et al.*, 2011). *E2Fa* is expressed during LR initiation, where it regulates the first asymmetric cell divisions (Berckmans *et al.*, 2011). Another *LBD* gene, *LBD16*, is activated in pairs of XPP cells during LR initiation, where it promotes polar nuclear migration, allowing the establishment of asymmetry in LRFCs and subsequent cell fate respecification (after the division) (Goh *et al.*, 2012*a*). In addition, *GLV6* transcription starts with the nuclear migration in LRFCs and its expression pattern coincides with *DR5* during LR initiation (Fernandez *et al.*, 2015). A proper GLV6 activity level in LRFCs is proposed to be important for the first asymmetric cell divisions, as lower GLV6 levels result in reduced levels of initiation events while increased GLV6 levels produce excessive anticlinal divisions (in XPP cells) and disturb the asymmetric pattern (Fig. 2B) (Fernandez *et al.*, 2015).

Independent of auxin signaling, the nuclear protein ABERRANT LATERAL ROOT FORMATION4 (ALF4) is required to maintain XPP cells in a mitosis-competent state needed for LR initiation (Fig. 2B) (DiDonato *et al.*, 2004; Dubrovsky *et al.*, 2008). In loss-of-function *alf4-1* mutant roots, *DR5* expression is still activated in patches of XPP cells, indicating that LRFCs are activated and probably prepared for cell division (Dubrovsky *et al.*, 2008). However, lack of ALF4 halts subsequent cell divisions (Dubrovsky *et al.*, 2008). Moreover, components involved in positioning the cell division plane have been reported to be required during LR initiation, such as the microtubule cytoskeleton serving the protein KATANIN1 (KTN1) and the mitosis regulator α-group AURORA (AUR) kinases (Van Damme *et al.*, 2011; Marhavý *et al.*, 2016). Finally, overexpression of the mobile CLEL (also known as GLV and RGF) peptides CLEL6 and CLEL7 inhibits LR initiation by disturbing the asymmetry of the founder cell divisions, probably via an auxin-independent pathway (Fig. 2B) (Meng *et al.*, 2012).

Signaling inputs for cell fate respecification

Cell fate respecification needs to be accomplished along and/or after the first cell division to guide the subsequent formation of LRP. However, how this process is achieved remains an open question. Induced pericycle cell divisions by endodermal cell ablation (ECA) are frequently periclinal, instead of the normally occurring anticlinal divisions, and do not result in the formation of LRP (Marhavý *et al.*, 2016), indicating that cell fate respecification requires additional signaling inputs. Auxin treatment of ECA-treated pericycle cells can re-position the cell division plane back to anticlinal and allow primordium development (Marhavý *et al.*, 2016), suggesting that auxin signaling is involved in cell fate respecification. In line with this, induction of the cell cycle in the *slr* mutant background does not result in LR formation even though it can trigger rounds of cell divisions, indicating that cell fate respecification requires SLR-mediated auxin signaling (Vanneste *et al.*, 2005).

Lateral root outgrowth

We here refer to LR outgrowth as the process that controls both primordium patterning and growth mainly guided by developmental signals within the primordium itself, including signals to specify *de novo* LR tissues and meristem. LR outgrowth is divided into several stages according to the number of completely formed cell layers in the primordia (Fig. 2C) (Malamy and Benfey, 1997). After LR initiation, an incipient Stage I LRP is formed and is considered as a sign of LR outgrowth onset (Fig. 2C). Cell growth and subsequent rounds of anticlinal, periclinal, and tangential cell divisions are launched to establish a dome-shaped primordium that emerges as a LR at the final stage of LR outgrowth (Fig. 2C) (Lucas *et al.*, 2013; von Wangenheim *et al.*, 2016). Emerged LRs possess a fully functional meristem that is highly reminiscent of the primary root meristem, in which the quiescent center (QC; cells with low mitotic activities) and the stem cells surrounding it make up root tissues, forming the core of root meristem (Fig. 2C) (Scheres, 2007; Bennett and Scheres, 2010). So far, our knowledge on specific molecular regulatory networks for LR outgrowth is limited, as this process involves many factors also important for primary root growth.

Facilitated by recent advances in live plant cell imaging and image analysis, 3- and 4-D images have been used to precisely describe spatiotemporal LR outgrowth dynamics (Lucas *et al.*, 2013; von Wangenheim *et al.*, 2016). The first asymmetric cell divisions in LRFCs are stereotypic and tightly regulated, after which the central short cells define the core of the primordium and contribute to the majority of the primordium cell mass (von Wangenheim *et al.*, 2016). However, the second round of cell divisions in central short cells become less deterministic, as they can undergo anticlinal or periclinal divisions without a preferred order (von Wangenheim *et al.*, 2016). From the third round of the cell cycle onward, cell division orientation follows a non-stereotyped order (Lucas *et al.*, 2013; von Wangenheim *et al.*, 2016). But a few general rules for cell divisions can still be determined during LR outgrowth: (i) cells tend to divide following a geometric 'shortest-wall' principle; (ii) cells tend to alternate their division orientation between two consecutive divisions; and (iii) the

outer layer generated from periclinal divisions predominantly initiates new periclinal divisions prior to the inner layer (von Wangenheim *et al.*, 2016). The semi-stochastic choice of division plane orientation during LR outgrowth suggests that patterning is not dependent on a fixed order of oriented divisions, which is in line with the dominance of positional signaling over lineage-dependent development in other plant meristems (Pilkington, 1929; Sussex, 1989; van den Berg *et al.*, 1995, 1997). However, the primordium shape for a given stage, formed by these indeterministic cell divisions, is highly conserved (Lucas *et al.*, 2013; von Wangenheim *et al.*, 2016). The general change of primordium shape and growth axis is initially parallel to the shoot–root axis and then rotates by 90°, pointing toward the primary root surface (Lucas *et al.*, 2013; von Wangenheim *et al.*, 2016).

LRs are able to be formed from root segments containing LRP around Stage IV on the growth medium without additional auxin supply (Laskowski *et al.*, 1995). This indicates that an autonomous functional meristem has formed in primordia with as few as 3–5 cell layers, which precedes the recognizable meristem architecture at the final stages of LR outgrowth (Fig. 2C) (Laskowski *et al.*, 1995). At this developmental phase, QC marker expression is restricted to the stem cell niche (Goh *et al.*, 2016). The establishment of a *de novo* meristem tightly associates with a proper establishment of an auxin maximum in the primordium (Fig. 2C), which requires the re-localization of polar auxin efflux carrier PIN proteins, mainly PIN1 (Benková *et al.*, 2003; Péret *et al.*, 2009*a*; Marhavý *et al.*, 2011, 2014). During LR outgrowth, cytokinin is able to regulate the direction of auxin flow, and thereby modulates auxin distribution (Marhavý *et al.*, 2011, 2014). This process is achieved by cytokinin-induced PIN1 depletion at specific cellular domains through modulating PIN1 endocytic recycling, leading to a rearrangement of its membrane localization (Marhavý *et al.*, 2014). This selective sensitivity of PIN1 polar localization to cytokinin correlates with the degree of PIN protein phosphorylation (Marhavý *et al.*, 2011). In addition, cytokinin signaling is crucial for the inhibition of LR outgrowth in response to genotoxic stress (Davis *et al.*, 2016). DNA lesions induced by the radiomimetic reagent zeocin (DNA double-strand breaks) inhibits LR outgrowth, which is mediated by the transcription factor SUPPRESSOR OF GAMMA RESPONSE 1 (SOG1) (Davis *et al.*, 2016). This LR outgrowth inhibition is released in cytokinin biosynthesis/signaling mutants and cytokinin biosynthetic genes are up-regulated upon zeocin treatment (Davis *et al.*, 2016).

The auxin-regulated *PUCHI* gene, encoding an APETALA2-like transcription factor, is involved in the definition of primordium boundaries during LR outgrowth (Hirota *et al.*, 2007; Kang *et al.*, 2013). *PUCHI* is initially expressed in all of the primordium cells at early developmental stages before being gradually restricted to the peripheral margin of the primordium (Hirota *et al.*, 2007). In the loss-of-function *puchi-1* mutant, cells in the flanks undergo additional divisions along with the shoot–root axis from Stage III onward (Hirota *et al.*, 2007). Moreover, *PUCHI* functions downstream of *ARF7*/*ARF19* and co-acts with *LBD16* and *LBD18* to define primordium boundaries, as double and triple *puchi* and *lbd* mutants show additive phenotypes at early stages (Kang *et al.*, 2013). Another gene involved in defining primordium boundaries is *ARABIDOPSIS HOMOLOGUE OF TRITHORAX1* (*ATX1*), encoding a H3K4-histone methyltransferase that maintains a number of genes in an active state (Napsucialy-Mendivil *et al.*, 2014). In loss-of-function *atx1-1* mutant roots, additional anticlinal cell divisions are frequently observed in the flanks (Napsucialy-Mendivil *et al.*, 2014). Moreover, in *atx1-1* mutant primordia cell proliferation is compromised and tissue patterning also seems interrupted, as the expression of cell cycle and QC markers are altered, resulting in a prolonged outgrowth time and thereby a decreased LR density (Napsucialy-Mendivil *et al.*, 2014). ATX1-mediated regulation of LR outgrowth is independent of an auxin response gradient, as DR5 expression remains unchanged (Napsucialy-Mendivil *et al.*, 2014). AtMYB36 has been implicated in the control of LRP boundaries (Fernández-Marcos *et al.*, 2017). *AtMYB36* is transcribed in the cells surrounding LRP after Stage V, where it defines LRP boundaries by maintaining reactive oxygen species (ROS) homeostasis (Fernández-Marcos *et al.*, 2017). Loss-of-function *myb36* results in more cells in the central base of the primordium, thereby altering primordium width (Fernandez-Marcos *et al.*, 2017).

LR outgrowth requires intercellular communication between LRP and its surrounding tissues (Marin *et al.*, 2010; Gibbs *et al.*, 2014; Yu *et al.*, 2015). *AtMYB93* is expressed strongly, specifically, and transiently in the endodermal cells overlaying early LRP, where it negatively controls LR outgrowth progression. *myb93-1* mutant roots exhibit an accelerated outgrowth rate, in terms of primordium age (Gibbs *et al.*, 2014). Small RNAs, microRNA390 (miR390), trans-acting short-interfering RNAs (tasiRNAs), and miR156 are also involved in LR outgrowth (Marin *et al.*, 2010; Yu *et al.*, 2015). Auxin-responsive *MIR390* is specifically expressed in the basal part of LRP and the underlying vascular parenchyma cells, where it triggers the biogenesis of *TAS3*-derived tasiRNAs (Marin *et al.*, 2010). These tasiRNAs inhibit ARF2, ARF3, and ARF4 expression, thus facilitating LR outgrowth progression (Marin *et al.*, 2010). In turn, positive and negative feedback regulation of miR390 by the targeted ARFs ensure the proper miR390 expression pattern, thereby optimizing LR outgrowth in response to endogenous and environmental fluctuations (Marin *et al.*, 2010). Similarly, miR156 and its target genes *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE3* (*SPL3*), *SPL9*, and *SPL10* are auxin responsive and are involved in regulating developmental progression during LR outgrowth (Yu *et al.*, 2015).

Lateral root emergence

LR emergence demands mutual crosstalk between the growing primordium and its overlaying tissues, including the endodermis, cortex, and epidermis. On its way to emergence, the LRP first encounters the endodermis tightened by the Casparian strip, the ring-like supracellular hydrophobic

impregnation between endodermal cells that sets up an apoplastic diffusion barrier (Naseer *et al.*, 2012; Lee *et al.*, 2013*b*; Vermeer *et al.*, 2014). During outgrowth of the primordium, the shape of overlaying endodermal cells undergoes a dramatic change (Vermeer *et al.*, 2014). These cells become progressively flattened and undergo cell volume loss until reaching the point where the plasma membrane from both sides (radially inner and outer) fuse without compromising membrane integrity, causing cell death (Vermeer *et al.*, 2014). Meanwhile, the integrity of the Casparian strip network is largely preserved, with only local degradation, to consolidate diffusion barriers in the emerging margins between the primordium and endodermis (Vermeer *et al.*, 2014; Vermeer and Geldner, 2015). This preservation of the Casparian strip during LR emergence guarantees the isolation of the vascular bundle from the outside (Vermeer *et al.*, 2014; Vermeer and Geldner, 2015). Eventually, a localized opening in the endodermal cell layer is formed, allowing the penetration of the primordium at Stages III–IV (Fig. 2C) (Kumpf *et al.*, 2013; Vermeer *et al.*, 2014).

Unlike endodermal cells that are tightly connected by the Casparian strip, cortical and epidermal cells barely change shape, but instead are pushed away by a loss of cell-to-cell adherence during LR emergence (Laskowski *et al.*, 2006; Péret *et al.*, 2009*b*; Kumpf *et al.*, 2013; Vermeer and Geldner, 2015). Successful emergence from the cortical and epidermal cells is accompanied with a fast 'dome-shaped' transition of the growing primordium at Stages V–VII (Fig. 2C) (Kumpf *et al.*, 2013; Lucas *et al.*, 2013; von Wangenheim *et al.*, 2016). This shape change seems independent of increased cell proliferation, and probably results from radial cell expansion due to release from the physical constraints of the overlaying tissues (Lucas *et al.*, 2013; von Wangenheim *et al.*, 2016). Auxin transport and signaling control the emergence from the cortex and epidermis (Marchant *et al.*, 2002; Swarup *et al.*, 2008; Péret *et al.*, 2013; Porco *et al.*, 2016). The auxin influx carrier AUX1 facilitates shoot-derived IAA loading to the vascular transport system, through which the aerial source of auxin necessary for LR emergence is channeled via LRFCs and/or primordia to their overlaying tissues even earlier than, or along with, LR initiation (Fig. 2C) (Marchant *et al.*, 2002; Swarup *et al.*, 2008; Péret *et al.*, 2013). At later developmental stages, auxin is channeled from the primordium apex to the overlaying tissues (Swarup *et al.*, 2008). Spatially precise induction of the auxin influx carrier LIKE-AUXIN3 (LAX3) in overlaying cortical and epidermal cells reinforces auxin-dependent induction of selective cell wall remodeling genes that promote cell separation (Swarup *et al.*, 2008). Circumferential *LAX3* expression is often restricted to two adjacent cortical cell files to ensure that cell separation occurs solely from their shared walls (Péret *et al.*, 2013). The sequential induction of *PIN3* and *LAX3* is required to stabilize the *LAX3* expression pattern in varied tissue geometries and auxin signaling levels (Péret *et al.*, 2013). Moreover, *LAX3* induction is auxin dependent and requires auxin SLR-ARF7 signaling pathways (Fig. 2C) (Swarup *et al.*, 2008; Lavenus *et al.*, 2015; Porco *et al.*, 2016). LBD29, a direct ARF7 downstream transcription factor expressed in the overlying tissues prior to *LAX3* activation (before LR initiation), functions as a direct positive *LAX3* upstream regulator (Fig. 2C) (Okushima *et al.*, 2007; Porco *et al.*, 2016). Sequential LBD29 and LAX3 induction by auxin is required to co-ordinate cell separation during LR emergence (Porco *et al.*, 2016). Other SLR-ARF7/ARF19 downstream regulators are also involved in LR emergence (Kim and Lee, 2013; Lee *et al.*, 2013*a*, 2014, 2015; Lee and Kim, 2013). LBD18 acts downstream of LAX3 to control LR emergence through up-regulating a subset *EXPANSIN* (*EXP*) genes, which encode non-hydrolytic cell wall loosening factors, including *EXP14* and *EXP17* (Kim and Lee, 2013; Lee *et al.*, 2013*a*, 2015; Lee and Kim, 2013). In addition, GFB INTERACTING PROTEIN1 (GIP1) interacts with LBD18 as a coactivator to enhance the transcriptional activation of *EXP14* by LBD18 (Lee *et al.*, 2014).

Other signals involved in modulating cell wall loosening and cellular osmotic pressure are important during LR emergence (Kumpf *et al.*, 2013; Orman-Ligeza *et al.*, 2016; Péret *et al.*, 2012). Cell separation of the overlaying tissue initiates from cell wall loosening, which is facilitated by the induction of cell wall degradation enzymes, particularly polygalacturonases (PGs) to hydrolyze pectin, the major component of plant cell walls (Kumpf *et al.*, 2013). The INFLORESCENCE DEFICIENT IN ABSCISSION (IDA) peptide signals via the leucine-rich repeat receptor-like kinases HAESA (HAE) and HAESA-LIKE2 (HSL2) in the overlaying tissues during emergence (Fig. 2C) (Kumpf *et al.*, 2013). In loss-of-function *ida*, *hae*, and *hsl2* mutant combinations, LRP penetration through the overlaying cells is impeded in cell separation (rather than LR outgrowth), which is mainly due to inefficient pectin degradation of the middle lamella, a pectin-based layer cementing the walls of adjacent cells (Kumpf *et al.*, 2013). Indeed, IDA, HAE, and HSL2 are all involved in regulation of cell wall remodeling genes, including *PG*s, during LR emergence in an auxin-mediated manner, as their expression in the overlaying tissues and auxin responsiveness requires intact auxin transportation (LAX3) and auxin signaling (SLR-ARF7/ARF19; Fig. 2C) (Laskowski *et al.*, 2006; Kumpf *et al.*, 2013).

Water flow between LRP and their overlaying tissues controls LR emergence (Péret *et al.*, 2012; Vilches-Barro and Maizel, 2015). During LR outgrowth, primordia at later developmental stages (older than Stage II) are symplasmically isolated from the vasculature as a result of enhanced callose deposition (Benitez-Alfonso *et al.*, 2013). Therefore, water influx into LRP requires aquaporins, the plasma membrane channels that facilitate water movement (Péret *et al.*, 2012). Aquaporins fall into several subfamilies, including PLASMA MEMBRANE INTRINSIC PROTEINS (PIPs) family, most of which respond negatively to auxin (Péret *et al.*, 2012). By combining mathematical modeling and experimental validation (using *pip2;1* mutants), a water reflux model in the overlaying tissues, based on the cell hydraulic conductivity, has been proposed to be critical during LR emergence. In this model, water uptake is repressed in the overlaying tissues while water transport from the overlaying tissues to the primordium is promoted (Péret *et al.*, 2012; Vilches-Barro and Maizel, 2015). This mechanism dynamically depletes

water in the overlaying tissues during LR emergence, thereby decreasing their resistance to the expansion of growing primordia (Péret *et al.*, 2012). Auxin negatively regulates aquaporin-dependent water transportation in tissues overlaying LRP (Péret *et al.*, 2012). It has been reported recently that the circadian clock is rephased during LR outgrowth (Voß *et al.*, 2015). The circadian clock is able to regulate aquaporin expression in other root tissues, resulting in a diurnal oscillation in root water uptake (Takase *et al.*, 2011; Voß *et al.*, 2015). By rephasing the circadian clock, LRP (and surrounding tissues) might be able to independently regulate their hydraulic properties in a manner distinct to other root tissues to facilitate organ emergence (Voß *et al.*, 2015). In line with this, mutants either lacking or overexpressing core clock components exhibit LR emergence defects; moreover, the circadian clock also controls the level of auxin and auxin-related genes involved in LR emergence, including *IAA14* and auxin oxidase *AtDAO2* (Voß *et al.*, 2015).

ROS have been reported to function as important signals during LR emergence (Orman-Ligeza *et al.*, 2016). ROS treatment is able to bypass the requirement of auxin influx carrier AUX1 and LAX3 activities to induce LR emergence in *aux1lax3* double-mutant roots, probably through triggering wall acidification in the overlaying tissues to facilitate cell wall remodeling (Orman-Ligeza *et al.*, 2016). ROS are detected in the middle lamella of overlaying endodermal and cortical cell walls that flank the growing primordium (Orman-Ligeza *et al.*, 2016). ROS donor *RESPIRATORY BURST OXIDISE HOMOLOGS* (*RBOH*) genes respond positively to auxin and spatiotemporally regulate ROS production in the overlaying tissue to facilitate LR emergence (Orman-Ligeza *et al.*, 2016).

General conclusions and perspectives

Significant advances have been made recently in the dissection of the molecular and genetic components involved in arabidopsis LR formation. In each developmental phase, auxin plays important roles. Exciting new insights have revealed underlying mechanisms in LR patterning, particularly pre-patterning, and LR emergence. An endogenous periodic clock associated with auxin-response oscillations continuously produces organogenesis-competent cells in the root tip during root growth. One biological explanation offered for this on-and-off switching mechanism for competence is that it might be a way to regulate the trade-off between resource investment and response time under changing environments (Van Norman *et al.*, 2013). The oscillation could also be important as a pattern-generating mechanism *per se*. It is still not known how intrinsic and extrinsic signals specify an exactly defined site for LR initiation, but the local accumulation of specific auxin responses in founder cells is an important aspect of this specification process. Other mechanisms that are able to turn XPP cells (without 'competence') into LR initiation sites upon drastic environmental stimuli might exist in parallel to the pre-patterning mechanism. Both scenarios can potentially explain the immense developmental plasticity of LR positioning. LR emergence is a remarkable model to study intercellular communication and co-ordination during organogenesis, greatly helped by new observations that describe the complexity of biomechanical and biochemical interactions between the growing primordium and its surrounding tissues (Vilches-Barro and Maizel, 2015). Also at these late stages, auxin signaling fulfills specific roles. Despite the progress made in LR pattering and emergence, our knowledge of key steps during LR initiation and outgrowth is still limited, thereby raising several open questions: (i) how the asymmetry in LRFCs is established; (ii) how the new cell identity is specified in the daughter cells along with and/or after the first cell divisions during LR initiation; and (iii) how *de novo* LR tissues and meristems are spatiotemporally formed. Recently, rapid progress has been made to dissect gene regulatory networks during LR formation based on large transcriptomic data sets, such as VisuaLRTC and TDCor (Parizot *et al.*, 2010; Lavenus *et al.*, 2015). The predicted network topology may facilitate the dissection of molecular networks underlying LR formation (De Rybel *et al.*, 2010). Moreover, modeling and advanced 4-D imaging techniques provide more accurate means to couple dynamic LR formation and gene regulatory networks, and also to explore this process from a biophysical perspective (Laskowski *et al.*, 2008; Péret *et al.*, 2013; Barbier de Reuille *et al.*, 2015; von Wangenheim *et al.*, 2016). In the future, a combination of these new approaches with traditional experimental studies will need to provide a more detailed understanding of the dynamic regulatory networks and the precise roles of auxin signaling in different contexts in order to unravel the processes that control LR development in arabidopsis and other plant species.

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