Yalamanchili *et al. Biology Direct* (2024) 19:87
https://doi.org/10.1186/s13062-024-00535-5

Biology Direct

REVIEW

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Shaping root architecture: towards understanding the mechanisms involved in lateral root development

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Abstract

Plants have an amazing ability to adapt to their environment, and this extends beyond biochemical responses and includes developmental changes that help them better exploit resources and survive. The plasticity observed in individual plant morphology is associated with robust developmental pathways that are influenced by environmental factors. However, there is still much to learn about the mechanisms behind the formation of the root system. In Arabidopsis thaliana, the root system displays a hierarchical structure with primary and secondary roots. The process of lateral root (LR) organogenesis involves multiple steps, including LR pre-patterning, LR initiation, LR outgrowth, and LR emergence. The study of root developmental plasticity in Arabidopsis has led to significant progress in understanding the mechanisms governing lateral root formation. The importance of root system architecture lies in its ability to shape the distribution of roots in the soil, which affects the plant's ability to acquire nutrients and water. In Arabidopsis, lateral roots originate from pericycle cells adjacent to the xylem poles known as the xylem-pole-pericycle (XPP). The positioning of LRs along the primary root is underpinned by a repetitive pre-patterning mechanism that establishes primed sites for future lateral root formation. In a subset of primed cells, the memory of a transient priming stimulus leads to the formation of stable pre-branch sites and the establishment of founder cell identity. These founder cells undergo a series of highly organized periclinal and anticlinal cell divisions and expansion to form lateral root primordia. Subsequently, LRP emerges through three overlying cell layers of the primary root, giving rise to fully developed LRs. In addition to LRs Arabidopsis can also develop adventitious lateral roots from the primary root in response to specific stress signals such as wounding or environmental cues. Overall, this review creates an overview of the mechanisms governing root lateral root formation which can be a stepping stone to improved crop yields and a better understanding of plant adaptation to changing environments.

Background

Plants have evolved remarkable flexibility to navigate dynamic changes in their biotic and abiotic environment. These adaptations extend beyond biochemical responses and include developmental changes, enabling plants to better exploit resources and ensure optimal performance and survival within its distinct microenvironment. The constant perception of variations in light, temperature, and nutrient availability prompts plants to modify characteristics, including the timing of developmental transitions (e.g., germination and flowering), as well as the number, growth rate, and branching pattern of organs.

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The plasticity observed in individual plant morphology is associated with robust developmental pathways. This is evident in the precise patterning systems governing organ development, which are influenced by environmental factors. For instance, the transcription factor mediated induction cascade of flower identity genes is coupled to multiple environmental sensing pathways for the orchestration of flowering in space and time [13, 15, 20, 47, 160]. While considerable progress has been made in understanding above-ground adaptations, there remains a significant gap in our understanding of root developmental plasticity. On one hand, the presence of developmentally robust, cell-type specific patterning mechanisms ensures the precise positioning of primary root and secondary root primordia. On the other hand, developmental plasticity allows for the initiation and growth of new root system branches in response to multiple endogenous and environmental inputs. These mechanisms and responses in root system architecture are crucial for the plants to explore and optimize resource acquisition under complex and heterogenous soil environments [26, 69, 90].

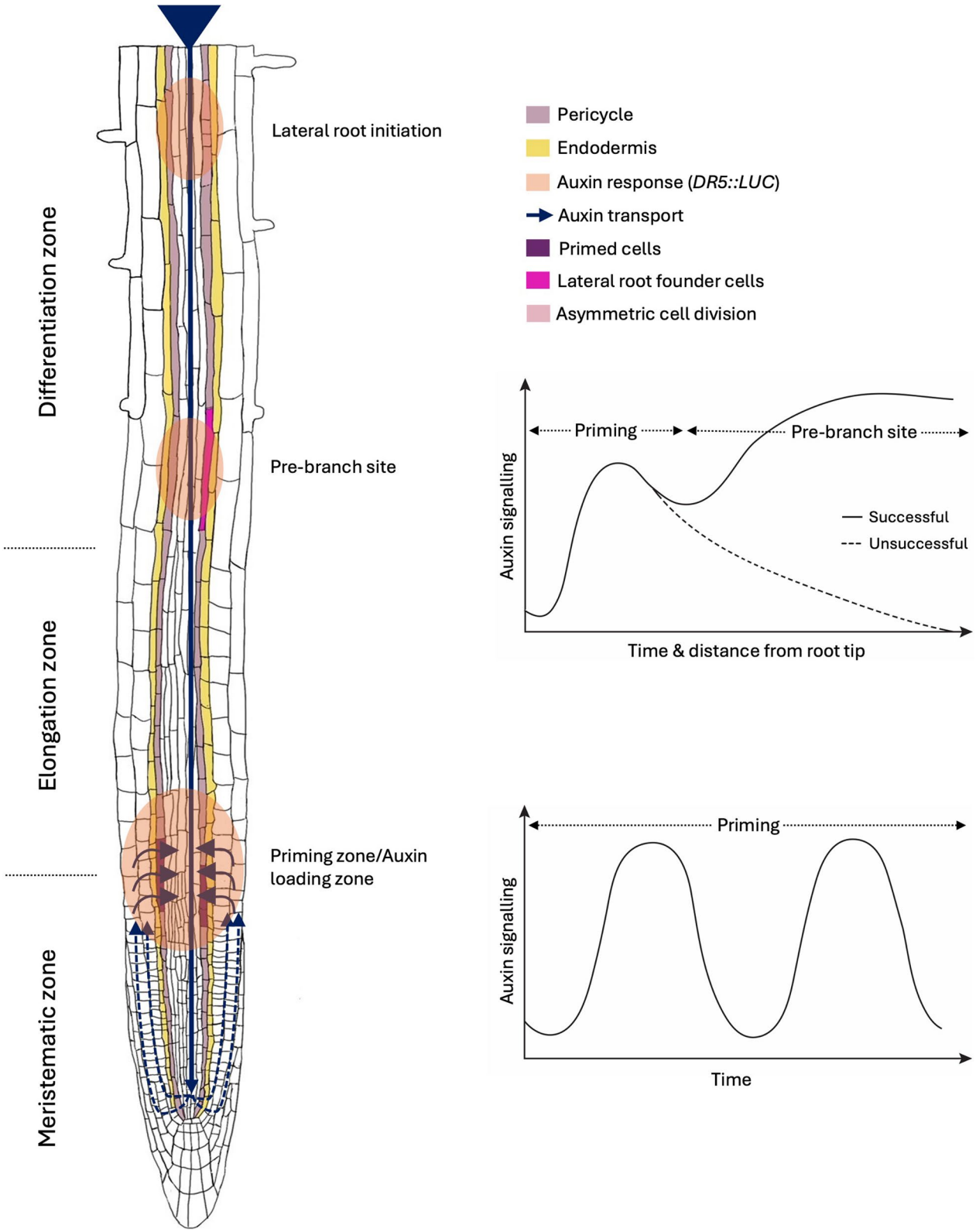
The root system of the dicotyledonous model plant *Arabidopsis thaliana* (*Arabidopsis*) displays a hierarchical structure with an embryonically formed primary root and post-embryonically formed secondary (lateral roots and adventitious lateral roots) and higher-order roots [1, 54, 116, 132]. These roots are radially organised into single concentric rings of epidermis, cortex, endodermis, and pericycle cells enclosing the central vasculature. At the distal end of root tips, the epidermis is enveloped by the root cap, playing a dual role in sensing and transmitting external stimuli as well as protecting the meristematic cells within the growing tip (Fig. 1) [34, 81]. The developmental trajectory of new cells that are continuously produced in the root apical meristem involves their shootward displacement through four longitudinal zones, defined based on cell proliferation and growth characteristics. Successively, cells progress from the meristematic zone, characterized by active cell division, to the transition zone with limited cell division and slow cytoplasmic cell growth. Cells then enter the elongation zone, marked by rapid cell elongation through vacuolar growth and finally they enter the differentiation zone, where cell growth terminates, and they acquire specialized identities (Fig. 1) [34, 86, 152].

Secondary roots play a significant role in shaping the root system architecture of dicot plants, making it a subject of considerable importance for agricultural improvement [26, 79]. In Arabidopsis, lateral roots (LRs) originate from pericycle cells adjacent to the xylem poles, known as the xylem-pole-pericycle (XPP), which exhibit long-term cell division potential [7, 86, 114]. The process of LR organogenesis in Arabidopsis involves multiple steps, including LR pre-patterning (LR priming and LR founder cell specification), LR initiation, LR outgrowth, and LR emergence [36]. The positioning of LRs along the primary root is underpinned by a repetitive pre-patterning mechanism that establishes primed sites for future lateral root formation [23]. In a subset of primed cells, the memory of a transient priming stimulus leads to the formation of stable pre-branch sites and establishment of founder cell identity (Fig. 1) [148]. These founder cells undergo a series of highly organized periclinal and anticlinal cell divisions and expansion to form lateral root primordia (LRP). Subsequently, LRP emerges through three overlying cell layers of the primary root, giving rise to fully developed LRs (Fig. 2) [92, 94, 116, 137, 157].

In addition, Arabidopsis can also develop adventitious lateral roots from the primary root, in response to specific stress signals such as wounding or environmental cues [2, 96, 132]. The plasticity of root development is underscored by the modulation of initiation and outgrowth frequency of secondary roots, along with the regulation of growth rates across various branches of the root system. In Arabidopsis, several regulatory networks and cellular mechanisms governing root development have been identified [4], [36, 102, 107, 137, 144]. Nevertheless, the integration of external inputs that induce plasticity into the developmental pathways orchestrating the root developmental program remains to be fully understood. This requires a comprehensive understanding of the basic developmental processes underlying LR formation, as a crucial first step towards unravelling the adaptive responses of roots. In the following sections, we delve into the key molecular and cellular mechanisms of

(See figure on next page.)

Fig. 1 Schematic overview of lateral root pre-patterning along the primary root. The formation of new LRs begins with priming of a subset of xylem pole pericycle cells in the basal meristem, which includes the transition zone and early elongation zone (priming zone). This priming process is characterized by periodic variations in cellular auxin signalling within the protoxylem cells, which is subsequently passed to the adjacent pericycle cells. As the primed pericycle cells are displaced during root growth, their auxin signalling decreases. The successful memorization of this transient priming signal leads to the formation of stable pre-branch sites in the differentiation zone, marked by a re-increase in auxin signalling. A subset of pericycle cells within these pre-branch sites acquires founder cell identity, initiate lateral root formation, and eventually develop into new emerged lateral roots



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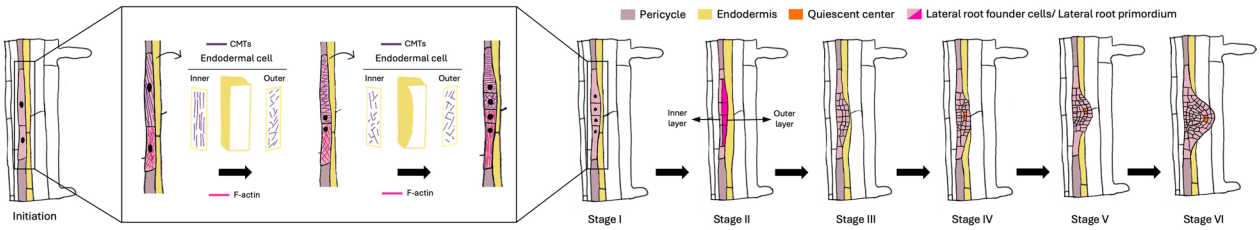

Fig. 2 Key cellular and morphological changes during lateral root initiation and development. The first visible step in lateral root morphogenesis involves the asymmetric expansion of lateral root founder cells (LRFCs) and the migration of their nuclei toward the common cell wall. This is followed by an asymmetric anticlinal division, resulting in the formation of a stage I lateral root primordium (LRP). Auxin signalling within the LRFCs and endodermis, along with localized rearrangements of cortical microtubules (CMTs) and actin microfilaments, play crucial roles in facilitating the asymmetric radial expansion and division of LRFCs. The establishment of the quiescent center occurs during the developmental phase transition of the LRP at stage IV, coinciding with the transition from a flat-topped to a dome-shaped primordium as it crosses the endodermis. LRP finally breaks through the overlying cortex and epidermis cells to emerge from the main root

LR development, with emphasis on early steps controlling pre-pattering and initiation of LRs.

Lateral root priming

In Arabidopsis, all pericycle cells retain the ability to form LRs, but only a subset of XPP cells, regularly spaced along the primary root, will become founder cells. This pre-patterning event known as priming occurs in the basal meristem of the root, comprising the transition zone and the early elongation zone (hereafter referred to as priming zone) (Fig. 1) [7, 23, 37, 106, 114]. LR priming is characterised by a periodic change in auxin responses and gene expression and leads to specification of a subset of cells competent for future LR formation (Fig. 1) [23, 106]. LR priming can be visualized using synthetic auxin signalling promoter activity, DIRECT REPEAT5 (*DR5*), which marks the auxin transcriptional response. In the priming zone, a recurring expression of the DR5:GUS reporter, marking the auxin signal maximum, is observed in the protoxylem files neighbouring the pericycle cells [23]. This auxin maximum was followed by periods of low auxin response, and the occurrence of auxin response peaks was shown to correlate with sites of future LR initiation [23]. Real-time analysis using the *DR5::Luciferase* (LUC) reporter substantiated these observations, revealing a repetitive pattern of DR5 expression near the root tip [106]. While the *DR5::LUC* reporter offers the advantage of being non-destructive, making it suitable for time-lapse imaging, it lacks cellular resolution. Nevertheless, studies using the DR5:GUS reporter have reported that the priming signal is initially observed in protoxylem and is subsequently transmitted to the overlying pericycle cells [23].

Various hypotheses have been proposed to explain the periodic generation of primed sites. In addition to the periodic variation in DR5 expression, a large number of number of genes were reported to oscillate either in phase (2084 genes) or antiphase (1409 genes) with DR5 [106]. This observation led to the hypothesis that, in addition to variation in auxin response, periodic changes in the expression of these oscillating genes might also play a crucial role in LR priming. However, not all auxin-responsive genes exhibited periodic expression in the priming zone. Among the oscillating genes, certain ones encoding transcription factors such as AUXIN RESPONSIVE FACTOR (ARF) 7 and LATERAL ORGAN BOUNDARIES DOMAIN (LBD) 16, SHATTERPROOF (SHP) 1 exhibited periodic behaviour in their expression over time. And mutants in some of the oscillating transcription factors from the MADS-box protein family (SHP1, SHP2, and SEEDSTICK), ARF (ARF7 and ARF2) and NAC family (VND2, FEZ, and SOMBRERO) resulted in reduction in the number of pre-branch sites and LRs. Based on these observations, an endogenous clock-type oscillator, akin to the segmentation clock in vertebrates, was proposed to drive the repetitive priming. The clock-and-wavefront mechanism in vertebrates is a well-established mechanism for the initiation of periodic gene expression propagating through a growing axis and ultimately resolving in a stable specification of somite boundaries [28, 106, 122]. However, despite this reported temporal oscillation in expression of thousands of genes in the priming zone [106], experimental support for a genetic oscillator resembling somitogenesis is lacking. On the other hand, there is growing evidence supporting the significance of auxin biosynthesis, transport, and signalling in triggering priming (Reviewed in detail in [48]). Additionally, studies have also revealed a strong positive correlation between priming and root growth [72, 121]. Van den Berg et al. [147] studied the role of root tip auxin transport and growth dynamics in driving the process of LR priming and compared this to the root-clock hypothesis. A multi-scale root growth model that incorporated root tip auxin transport dynamics, root

developmental zonation, and root growth dynamics was developed which allowed to simultaneously monitoring auxin dynamics at various levels, while independently altering various aspects of auxin and growth dynamics in the model. This revealed that LR priming is an emergent property driven by a reflux-and-growth mechanism. It was demonstrated how the interplay between the auxin reflux loop establishing the auxin loading domain in the priming zone, and the root growth dynamics periodically generating pairs of large and small cells within this domain leads to preferential auxin loading in the larger cells, giving rise to periodic variations in auxin levels. In addition, these observations establish a significant positive correlation between priming frequency and priming site spacing with the root meristem growth, as predicted by the reflux-and-growth mechanism. This finding challenges the validity of the earlier proposed Turing and clock-and-wavefront models, which only explain one of these relationships but not both, whereas the reflux-and-growth mechanism addresses both. Recently, Goh et al., [60] developed a motion-tracking confocal microscope, combined with a deep learning and genetic algorithm assisted image processing, that enabled semi-automated quantification of cortical cell division and elongation dynamics at the root tip. Notably, this approach revealed cluster-constrained cell size alternation in root cortex cells, validating the cell expansion and division dynamics proposed in the reflux-and-growth model. This technological advancement offers a potential solution to the challenge of simultaneously monitoring transient cell size differences and auxin dynamics and provide better insights into the intricate cellular processes underpinning LR priming. The role of cell size differences in auxin-based patterning, as highlighted by the reflux-and-growth mechanism, is consistent with previous research demonstrating that an increase in cell size on the outer curve of the root bend raises auxin levels in these cells, leading to a positive feedback loop driving further increase in auxin levels and subsequently triggering LR formation [84].

Furthermore, it has been suggested that the root cap could play a critical role in regulating priming amplitude. Xuan et al. [25] demonstrated that the conversion of the auxin precursor indole-3-butyric acid (IBA) to indole-3-acetic acid (IAA) in the root cap creates an auxin source, that contributes to the priming amplitude and determines its successful translation into a pre-branch site. Analysing the priming dynamics in the mutants defective in conversion of IBA to IAA displayed normal periodicity of DR5 expression. However, DR5 signal intensity was dampened, considerably affecting the pre-branch site formation [25]. The IBA to IAA conversion primarily occurs in the outer lateral root cap cells and it is then transported to the priming zone [22, 25, 121]. This transport is closely associated with the periodic programmed cell death (PCD) in the lateral root cap cells. As these cells reach the onset of the elongation zone, the DR5rev:VENUS-N7 expression in the most distal lateral root cap cells fades periodically just before apoptosis. Auxin released from the cells undergoing apoptosis is suggested to be taken up the neighbouring epidermal cells and transferred to the site of LR priming. The periodicity of the PCD and DR5 expression are spatiotemporally correlated showing that PCD timing is coupled to the frequency of pre-branch site formation [121]. In *smb-3*, a loss-of-function mutant in SOMBRERO, delayed PCD of the lateral root cap cells, results in additional lateral root cap layers [11, 100]. The periodicity and regularity of LR priming are affected in *smb-3,* subsequently reducing the number of pre-branch sites and LRs [121]. These correlations between lateral root cap maturation and priming connect the two processes and is in line with the reflux-and-growth mechanism. The central role of growth in priming uncovered by Van den Berg et al. [147], suggest that the previously observed correlation between lateral root cap apoptosis and priming events [121] can be attributed to the coordination between root cap apoptosis and meristem growth dynamics, implying that synchronized growth processes, rather than lateral root cap shedding alone, contribute to LR priming. Notably, this also aligns with the observation that LR formation is suppressed in the absence of light, as light is known to affect the root growth dynamics [72], further supporting the role of growth dynamics in LR development.

Perhaps not surprisingly due to the strict correlation with auxin response as a measure of priming, genes involved in auxin transport and response pathway are reported to affect the frequency and amplitude of priming signal. In both loss-of-function mutant, *arf7-1* and gain-of-function mutant *iaa18/potent, DR5::LUC* expression in the priming zone was increased and it lacked normal periodic expression pattern, affecting pre-branch site formation. It was also reported that the IAA18/POTENT-ARF7 module influences the periodicity of the priming by activating and repressing the in-phase and antiphase genes, respectively suggesting they play a role in the oscillatory network. [106, 119]. Successful formation of pre-branch sites is determined by the priming amplitude. In *transport inhibitor response1/auxin signalling f-box protein (tir1afb2)* double mutants, auxin signal transduction was strongly reduced, leading to a significant reduction in the priming amplitude and pre-branch site formation without altering the priming frequency [25]. Similarly, loss of function of an influx carrier AUX1, reduced auxin levels in the lateral root cap and epidermal

cells, as well as DR5:GUS expression in the basal meristem of the roots [23]. However, *pin-formed (pin) 2* and *pin2 abcb1 abcb19* mutants only had mild effects on the LR formation possibly due to functional redundancy [121]. Since these genes influence auxin production, transport, and perception, the effect on LR priming observed in these studies could likely be indirect. Also, in line with the reflux-and-growth model, mutations that affect either auxin production or transport are predicted to decrease oscillation amplitude, consequently leading to reduced priming efficacy. Similarly, mutations that influence primary root growth or modify meristematic cell sizes are also predicted to diminish oscillation amplitude, which is fully consistent with the observed reduction in pre-branch sites and LR numbers in these studies [23, 25, 31, 89, 119, 139, 141].

While auxin and auxin signalling play a central role in LR priming, auxin alone is not sufficient to specify a pre-branch site. Localized exogenous auxin treatments in the priming zone could not specify new pre-branch sites but shifted the location of pre-branch site when the application was concurrent with a natural peak of DR5 expression. In addition, root gravitropic responses were found to affect the frequency of priming, transiently shortening the interval of DR5 expression but they did not affect the pre-branch site specification [106, 121].

In addition, non-auxin molecules, such as carotenoids, have been reported to influence LR priming. Inhibiting carotenoid biosynthesis genetically or with chemical treatments modulated priming and affected LR formation. Seedlings treated with compounds like CPTA (2-(4-chlorophenylthio)-triethylamine hydrochloride), which inhibit key enzymes in the carotenoid biosynthesis pathway, resulted in aberrant priming periodicity, as well as fewer pre-branch sites and LRs. A synthetic carotenoid cleavage inhibitor, D15, also reduced priming amplitude through an uncharacterized carotenoid-derived molecule that functions non–cell-autonomously. A similar reduction in LR capacity was also observed in mutants in the carotenoid biosynthesis genes, CHLOROPLAST BIOGENESIS 6 and PHYTOENE SYNTHASE (PSY). Interestingly, expression of PSY was excluded from the priming zone suggesting a non-cell autonomous function [149]. The endogenous apocarotenoid, β-cyclocitral, was shown to rescue the D15-induced LR phenotypes. However, the effect was indirect, as it does not affect priming, instead induces cell divisions in primary and lateral root meristems [30]. Nevertheless, treatment with another endogenous metabolite, retinal, that was found to be decreased in D15 treated roots, rescued D15 inhibition of priming amplitude, LR formation, as well as cell elongation. Mutants in Temperature-induced lipocalin (TIL), a retinal interactor, showed reduced priming amplitude, LR branching and cell elongation phenocopying D15 inhibition, suggesting TIL could act as a potential link between carotenoid biosynthesis and LR priming [31]. However, a mechanism behind carotenoid-based regulation of LR priming is yet to be discovered.

While the regular priming pattern establishes LR competent sites in an acropetal sequence, it is important to note that not all competent sites develop into functional LRs. Subsequent LR development does not strictly follow the acropetal pattern due to various factors leading to developmental delays or arrests at different stages [37, 39]. These findings support the existence of a series of growth control checkpoints to govern and, when necessary, halt LR progression at specific developmental stages. Such regulation enables the plant to precisely tailor the extent and positioning of root branching, allowing it to respond effectively to both experimental cues and developmental requirements. By orchestrating the initiation of organ formation and strategically imposing dormancy at various stages, this approach potentially optimizes the allocation of the plant's resources and its responsiveness to varying environmental conditions. Identification of genes involved at each of these checkpoints is progressively defining the landscape of this developmental regulation.

Lateral root founder cell specification

Lateral root founder cell (LRFC) specification is the second pre-patterning event and represents the final step in the transition of XPP cell identity. LRFC specification can be genetically separated from the patterned cell division during primordium morphogenesis [40]. The transient increase in auxin responses observed during LR priming dissipates as the cells are displaced out of the priming zone. Successfully primed XPP cells retain a memory of the initial priming signal, later transforming it into stable pre-branch sites in the differentiation zone. This is characterized by secondary elevation in *DR5::LUC* expression that is sustained (Fig. 1) [21, 23, 106]. However, not all static points of *DR5::LUC* expression result in persistent pre-branch site formation. Instead, some non-persistent pre-branch sites only display a transient increase in auxin response that fades out prior to primordia formation [106, 143]. Due to lack of cellular resolution, *DR5::LUC* marks a broad domain, however only few pericycle cells participate in founder cell specification [148]. This subset of pericycle cells that have gained LRFC identity undergo subsequent activation of cell division, leading to primordia formation.

The establishment of pre-branch sites following priming is marked by a stable secondary increase in auxin signalling. A recent investigation employing a computational reverse-engineering approach indicated that the

stable memorization of the initial auxin elevation relies on the temporal integration of the auxin signal, likely facilitated by epigenetic modifications. This temporal integration not only allows for a robust distinction of primed cells within the pericycle but also enables selective auxin-induced upregulation of genes like AUXIN RESPONSE FACTORS (*ARF*s) within these primed cells. The resulting amplification in auxin signalling capacity effectively counteracts the growth-induced dissipation of auxin levels and, consequently, the memorization of auxin signalling in these primed cells [128]. These findings provide clarity on the separate roles played by auxin abundance and auxin signalling in the pre-patterning of LRs, emphasizing that the initial priming involves cell growth resulting in an elevation in a cellular auxin concentration, followed by priming-induced upregulation of auxin signalling that orchestrates the formation of stable pre-branch sites.

Recently, the regulation of carbon and energy signalling pathways has been connected to lateral root formation. Trehalose-6-phosphate (T6P) regulates root branching through master kinases SNF1-related kinase-1 (SnRK1) and Target of Rapamycin (TOR). This, in turn, involves auxin and leads to an increase in root branching by coordinating the inhibition of SnRK1 and the activation of TOR in lateral root founder cells. Auxin affects this T6P function by transcriptionally down-regulating the T6P-degrader trehalose phosphate phosphatase B. This reveals a regulatory energy-balance network for LR formation that links T6P to both SnRK1 and TOR downstream of auxin. TOR acts as a central gatekeeper for root branching that integrates local auxin-dependent pathways with systemic metabolic signals, thereby modulating the translation of auxin-induced genes [103, 136].

In recent years, a handful of genes have been identified to be specifically expressed in the LRFCs preceding the first formative cell divisions, serving as molecular markers for this cytologically indistinguishable phase of LR formation. These include GATA TRANSCRIPTION FACTOR 23 (*GATA23*), MEMBRANE-ASSOCIATED KINASE REGULATOR 4 (*MAKR4*), *LBD16*, TARGET OF LBD SIXTEEN 2 (*TOLS2*), GOLVEN (*GLV*) 6, *GLV10*, PLETHORA (*PLT*) *3, PLT5, PLT*7, RALF LIKE 34 (*RALFL34*) and the F-box protein S-Phase Kinase-Associated Protein (*SKP*) 2B [21, 25, 44, 46, 57, 70, 95, 108, 143]. Many of these factors are involved in controlling LR positioning as revealed by their effect on number and location of LRP/LRs in the mutant lines.

In XPP cells, an auxin transcriptional signalling involves the degradation of the repressor Aux/IAA28, leading to the subsequent activation of its interacting ARFs (ARF5, ARF6, ARF7, ARF8, and ARF19). This activation is crucial for the expression of the GATA23 transcription factor, which is detected not only in LRFCs but also in early stages of LRP [21]. Functional studies suggested a potential role for GATA23 as a founder cell specification factor. Knockdown and gain-of function lines of GATA23 resulted in significant decrease or increase in early primordia stages, respectively [21]. A small signalling peptide, RALFL34 acts genetically upstream of GATA23. Mutants in RALFL34 displayed an enrichment in the early-stage primordia, resembling the phenotype of GATA23 gain-of-function lines. However, as the lateral root initiation phenotypes of their mutants do not match, the regulation is likely indirect or potentially involves additional regulators [108]. Another early marker for founder cell identity is MAKR4, which acts downstream of IBA-to-IAA conversion in the root tip. MAKR4 expression is observed in the protoxylem pole of the meristem with low levels in the priming zone. Strong expression of MAKR4 is detected in the pre-branch sites, localised to the plasma membrane of LRFCs prior to the morphologically visible initiation events. However, knocking down MAKR4 resulted in fewer LRP without affecting the pre-branch site numbers [25]. Additionally, LBD16 was reported to act upstream of MAKR4 [59]. Both MAKR4 and LBD16 are auxin inducible and play a role in the developmental progression of pre-branch sites to LRP but the underlying mechanism remains to be elucidated [25, 57].

Epigenetic control of gene expression through chromatin modifiers has been shown to influence a plethora of developmental processes including LR formation [64, 66, 133, 158]. In Arabidopsis, Polycomb group proteins are well conserved chromatin regulators that are classified into Polycomb repressive complex (PRC) 1 and PRC2. Subunits of PRC2 complex, EMBRYONIC FLOWER (EMF)-PRC2 CURLY LEAF (CLF) and its partner EMF2 play an inhibitory role in the LRFC establishment. In addition to being expressed in presumptive LRFCs, CLF is also strongly expressed in basal meristem and directly represses the expression of the auxin transport protein PIN1 [64], which is crucial for establishing an auxin maximum in the root tip [126]. Accordingly, loss of CLF function causes an increase in the DR5 reporter activity in the root tips and pre-branch sites, likely causing multiple primed XPP cells to develop into founder cells, thereby increasing the rate of LR initiation [64].

Lateral root spacing

The regular spacing of lateral organs along the primary root is tightly regulated in Arabidopsis, and a number of candidate genes are involved in preventing the formation of multiple pre-branch sites close to one another. Under normal growth conditions, it is not uncommon to observe two closely located pre-branch sites along the

longitudinal axis, however a range of lateral inhibition process repress the development of nearby LRs in the vicinity of pre-existing primordia [9, 78, 143]. Cell-to-cell communication is likely a critical factor in LRFC establishment, as the lateral inhibition mechanism involves the co-ordination between existing founder cell and the adjacent pericycle cells. Symplastic intercellular transport mediated by plasmodesmata is dynamically regulated during the LR development. Connectivity between XPP (including LRFCs) and surrounding cells becomes gradually restricted as the LR develops, and ceases completely in primordia older than stage IV, correlating with increased callose deposition at plasmodesmata [9]. Manipulating symplastic connectivity by interfering with callose degradation in the *plasmodesmal-localized β-1,3-glucanase (pdbg) 1 pdbg2* double-mutants resulted in restricted plasmodesmatal transport and an increase in LR density with closely spaced pre-branch sites and LRP. Interestingly, impairing the symplastic communication in the mature XPP was sufficient to induce the initiation of neighbouring LRP in clusters. This underscores the importance of intercellular connectivity to restrict the LR initiation to the recruited founder cells, possibly through the movement of non-cell autonomous factors [9].

Furthermore, receptor-like protein kinases, capable of interpreting signals from apoplastic communication, also play a significant role in cell-to-cell communication [6, 27, 62, 156, 159]. ARABIDOPSIS CRINKLY4 (ACR4), a receptor-like kinase that is localised at plasmodesmata of the small daughter cells formed from the first asymmetric cell division of LRFCs, was shown to prevent the proximal pericycle cells from dividing [27, 135]. Single and higher order mutants of ACR4 and its family members produced LRPs at higher densities that are clustered or fused. These localisation and mutant phenotypes hint at the role of plasmodesmata dependent intercellular communication and ACR4-mediated lateral inhibition [27]. GLUTAMATE RECEPTOR-LIKE (GLR) 3.2 and GLR3.4 are preferentially expressed in the root phloem and are reported to reside in the plasma membrane. They function as amino acid gated Ca^{2+} channels to regulate the LR positioning as revealed by increased amounts of primordia, which are aberrantly positioned in the mutant lines [156]

A peptide-receptor complex TOLS2—RECEPTOR-LIKE KINASE7 (RLK7) was shown to regulate the lateral inhibition process in a non-cell-autonomous manner to constrain LR formation [143]. TOLS2 is expressed in LRFCs and LRP, it is a direct transcriptional target of LBD16 [143] and is therefore downstream of the IAA14-ARF7/ARF19 auxin signalling module, which plays an important role in LR initiation [57, 110]. Disrupting this pathway either through loss-of-function *rlk7* mutant or double mutant *tols2pip2* of TOLS2 and a close homolog PLASMA MEMBRANE INTRINSIC PROTEIN2 (PIP2) resulted in an increased number of pre-branch sites, often found in close proximity to each other. Analysis of *DR5::LUC* signal dynamics in wild-type roots revealed that, in regions where priming resulted in a pair of closely located pre-branch sites, only one of them sustained expression, and the other one was typically transient. In contrast, both sites frequently showed similar expression levels of *DR5::LUC* in *rlk7* mutant possibly due to defective lateral inhibition [143]. Furthermore, PUCHI was identified as a downstream component of the TOLS2–RLK7 signalling module. Accordingly, *puchi* mutants displayed an increased frequency of pairs of pre-branch sites. Intriguingly, the increase in pre-branch sites in *rlk7* mutant is not translated into increased LR density, as it does in *puchi* mutant indicating additional mechanisms are in place to regulate LR formation after the founder cell establishment, which might involve PUCHI [143]. Recently, another peptide-receptor module GLV6/GLV10—ROOT GROWTH FACTOR INSENSITIVE (RGI) was found to regulate LR spacing via a partially shared pathway with TOLS2–RLK7 signalling module [73]. Despite being phylogenetically unrelated, both modules exhibit high level of similarity in spatiotemporal expression patterns, gain- and loss-of-function phenotypes and induce similar transcriptional responses [44, 46, 73, 143]. In addition, the expression of GLV6, GLV10 and TOLS2 is induced by LBD16. GLV6/10-RGI and TOLS2-RLK7 signalling pathways converge in phosphorylation cascade of MITOGEN-ACTIVATED PROTEIN KINASE (MPK) 3/MPK6, leading to shared transcriptional response, including the upregulation of PLT5 and PUCHI, that are known regulators of LR initiation and spacing [73].

Additionally, several studies have reported clustering phenotype of LRP/LRs in a wide range of mutants, suggesting impaired lateral inhibition. In the weak loss-of-function mutant in MONOPTEROS(MP)/ARF5 and gain-of-function mutant in its inhibitor BODENLOS(BDL)/IAA12, LRs were closely spaced, in some cases, fused [24]. Similarly, three PLT (PLT3, PLT5, PLT7) transcription factors control the positioning of the LRPs. In, *plt3plt7* double mutant and *plt3plt5plt7* triple mutant, LRP were frequently clustered together along the longitudinal and radial axis [70]. Intriguingly, TOLS2-RLK7-PUCHI, GLV6/GLV10-RG, BDL-MP, and PLT3/PLT5/PLT7 act downstream of SLR/IAA14-ARF7-ARF19 auxin signalling module to regulate the LR positioning. However, additional studies are needed to determine if the components of this intricate network interact to control the proper spacing of LRs. Furthermore, mutants in auxin signalling and transport, such as mutants in auxin

efflux carriers—PIN proteins (*pin2pin3pin7)* [84], gain of function allele of IAA3/short hypocotyl2 (*shy2-101)* [56], gain/loss-of-function mutants of small signalling peptides and receptors, C-TERMINALLY ENCODED PEPTIDE5 [125], RALFL34 receptor THESEUS1 (*the1-1 and the1-4*) [62] also resulted in clustered/fused LR phenotypes.

The precise regulation of the synthesis and signalling of the phytohormone cytokinin also contributes to providing positional information for LRPs along the primary root [14, 17, 82]. The expression of several cytokinin metabolism and signaling genes, along with phenotype analysis of cytokinin-deficient plants, suggests that cytokinin acts as a paracrine signal in controlling LR formation through lateral inhibition. Cytokinin is synthesized in both LRFCs and their neighbouring pericycle cells, creating a local inhibitory gradient that supresses LR initiation in the vicinity of existing primordia [17].

While priming occurs symmetrically at both xylem poles in Arabidopsis, LRs rarely form at the same position on opposite xylem poles, implying symmetry breaking occurs during the LRFC specification stage (Fig. 1). When Arabidopsis seedlings are grown on the surface of an inclined agar plate, an interaction between gravitropic and thigmotropic responses creates a waving growth of the primary root [77, 142]. This waving growth is accompanied by the tendency of LRs to be positioned on the outside (convex side) of the resultant bend. In addition, mechanical and gravitropic stimuli also promotes positioning of LRs predominantly on the outer side of bending roots [33, 77, 84, 91, 124]. Such bending mediated LR positioning leads to long-lasting fixation of LR sidedness as auxin treatment also induces de novo LRs only on the outside of the curvature [77]. These findings also uncoupled the curvature mediated positioning of LRs from the overall frequency of LR formation.

So far two distinct symmetry breaking mechanisms have been proposed for the determination of LR sidedness i) curvature induced differences in cell geometry and resulting changes in local auxin accumulation [84] and ii) cytoplasmic Ca^{2+} changes during bending [124] are suggested to play a role in determining LR sidedness. A preferential increase in auxin accumulation was observed in slightly larger cells on the stretched, convex side of the bend favouring the site for LR initiation [33, 84]. Consistently, an outward bias in AUX1 expression and PIN protein reorientation lead to the observed auxin accumulation dynamics at the bend [33, 84]. However, several mutants defective in auxin transport and/or signalling still form LR on the convex side of the mechanically induced curves, indicating that competence to form a LR is still present in these mutants [33, 124]. In contrast, after a 20-s transient mechanical bending, LR initiation occurs on the convex side of the bent root without obvious changes in cell geometry, potentially operating through an auxin-independent mechanism. The tension on the stretched/convex side of the root bend elicited a rapid transient increase in cytosolic Ca^{2+} levels only on the convex side of the curve, suggesting that bend induced calcium signalling could play a role in determining LR sidedness [124]. Overall, our current understanding of signalling cascades underlying LRFC specification is fragmentary, and additional studies are necessary to understand the interdependence or the mutual exclusivity of the above-described regulators and/or mechanisms.

Lateral root initiation

Shortly after the establishment of founder cell identity, a cascade of cellular and molecular events ensues marking the initiation of LR morphogenesis. The first morphological sign of LR initiation is the alteration in nuclear morphology of longitudinally adjacent founder cells, transitioning from spindle to round shape, accompanied by nuclear migration towards their shared cell wall. Concomitant with polar migration of nuclei, LRFCs undergo asymmetric radial expansion ([5]). The central domain where the founder cells abut expands more than the distal regions, creating a dome shaped appearance [5, 38, 123]. To spatially accommodate these changes, locally overlying endodermal cells shrink and/or modify their shape [98, 154]. Subsequently, the founder cells undergo asymmetric anticlinal cell division, yielding two small daughter cells and two large flanking cells. This formative cell division results in the formation of stage I LRP and marks the completion of LR initiation (Fig. 2). The proper co-ordination and execution of each of these events during LR initiation are necessary for the correct development of LR [5, 123, 154, 157].

During LR initiation, the order in which founder cells are recruited has been studied using clonal analysis and time-lapse imaging [129, 157]. The findings suggest that LR initiation typically begins with single founder cell (unicellular) in most cases and occasionally from two longitudinally adjacent cells (bicellular). In unicellular LR initiation, the single founder cell undergoes a first anticlinal division to form a two-cell stage recently recognised as stage 0, which is atypical in bicellular LR initiation. Subsequently, an auxin-dependent process gradually recruits neighbouring pericycle cells in the transverse and longitudinal plane as founder cells [129]. This generates a morphogenetic field comprising of 4–8 transverse pericycle cell files among which the auxin response reaches a maximum in the central master file and progressively reduces towards the periphery. The central master file also has the highest impact on LR formation and contributes the largest number of cells to the

LRP. Hence, the observed level of auxin response in the founder cell files aligns with their proliferation capacity. It is also important to note that number of founder cells participating in LR initiation and the contribution of each founder cell to the primordium is variable between primordia, suggesting a considerable extent of plasticity in cell patterning during LR formation [129, 157]. Typically, the formation of the founder cell morphogenetic field is confined by pericycle cells near the phloem pole. However, these cells possess the potential to be recruited as founder cells, but they exhibit restricted proliferation capacity [129].

Prior to the initiation of the first asymmetric cell division, the polarity of LRFCs changes and they undergo anisotropic radial expansion which are hallmark features of LR initiation. Auxin-dependent remodelling of cytoskeleton dynamics in pericycle and endodermis are essential for the asymmetric swelling of LRFCs and polar migration of the nuclei [5, 49, 123]. During the radial expansion, cortical microtubules within founder cells undergo progressive reorganization into two distinct domains: an isotropic array in the central domain undergoing expansion and an anisotropic transversal array in peripheral domain with restricted expansion (Fig. 2). These dynamics are important for both enabling central domain expansion and restricting radial expansion in the peripheral domain, resulting in asymmetric swelling. Although the driver for differential growth between the central and distal regions is unclear, a feedback mechanism involving cortical microtubules is implicated in amplifying the growth difference [5]. The asymmetric expansion of founder cells is accompanied by nuclear rounding and polar migration of founder cell nuclei. The reorganization of the F-actin network is necessary for nuclear migration (Fig. 2), and this process is regulated by LBD-mediated auxin signalling. Perturbing cortical microtubule and F-actin cytoskeletal dynamics interferes with the asymmetric radial expansion and polar nuclear migration respectively, leading to symmetric divisions of founder cells ([5]). Additionally, EXPANSIN A1 (EXPA1), an enzyme involved in cell wall remodelling, contributes to the radial swelling of LRFCs, potentially by modifying the composition of the pericycle cell wall, thereby controlling its width and the positioning of first founder cell division [123].

The radial expansion of LRFCs is facilitated by the accommodation responses of overlying endodermal cells. These cells undergo volume loss and/or modify their shape to release mechanical constraints and enable the radial growth of LRFCs [98, 153, 154]. IAA-mediated auxin signalling and cortical microtubule reorganisation in the endodermis are required to accommodate the expansion of founder cells [138, 154]. Accordingly, interfering with IAA-mediated auxin signalling specifically in the endodermis by expressing the *shy2-2* stabilised form of auxin repressor SHORT HYPOCOTYL 2 (SHY2) in the differentiated endodermal cells abolishes endodermal remodelling, resulting in an early and complete block of LR initiation [154]. Furthermore, ablation of endodermal cells in this line is sufficient to overcome the mechanical resistance and activate pericycle cell division, albeit periclinally. The reorientation of ablation-triggered pericycle division plane from periclinal to anticlinal, requires cotreatment with auxin, suggesting that cell fate respecification is governed by auxin signalling. Furthermore, this auxin-driven reorientation also requires TIR1/AFB receptor-mediated perception and a dynamic microtubule cytoskeleton [98].

In differentiated endodermal cells, the cortical microtubule lattice on the inner side (in contact with pericycle) and on the outer side displays differential organization. The arrays on the inner side are more anisotropic (along the shoot–root axis) compared to those on the outer side of the cell. During LR initiation, to facilitate the endodermal remodelling in response to expanding founder cells, endodermal cortical microtubule arrays on the inner side reorient to become isotropic resembling the outer side (Fig. 2). IAA-mediated auxin signalling in endodermis is reported to be responsible for the spatiotemporal reorganisation of cortical microtubules on the inner side. Additionally, MICROTUBULE ASSOCIATED PROTEIN 70-5 (MAP70-5) also regulates the switch in endodermal cortical microtubule arrays from anisotropic to more isotropic and possibly function as an integrator of mechanical constraints during LR initiation. Loss-of-function mutants of MAP70-5 lack the initial differential organisation of endodermal microtubules, and the arrays on the inner side remain invariably isotropic, i.e., in a primed stated for LR initiation, thereby resulting in increased rate of LR initiation. Taken together, these studies demonstrate that a coordinated contribution from pericycle and endodermis is essential for facilitating LR initiation [138].

The developmental progression from LRFCs to LR initiation is rapid and occurs within the same developmental zone, i.e., the differentiation zone [41]. The first asymmetric division of the founder cells marking the start of LRP morphogenesis is tightly regulated and occurs 4–7 h after the onset of nuclear relocation and swelling of founder cells [21]. Key components of auxin signal transduction play a central role in this developmental progression as well as coordinating responses in overlying cell layers. During LR initiation, a well-established signalling pathway involving the SOLITARY-ROOT (SLR)/IAA14–ARF7–ARF19 module drives both polar nuclear migration in founder cells and their asymmetric

cell division. Auxin accumulation in the LRFCs triggers ubiquitin-mediated degradation of SLR/IAA14 repressor protein, thus releasing ARF7 and ARF19 transcription factors to activate a downstream transcriptional cascade [51, 52, 110, 134]. Mutations resulting in gain-of-function of SLR (*slr-1*) or loss-of-function of its interacting partners ARF7 and ARF19 (*arf7arf19*) create a cell cycle arrest, subsequently blocking formative divisions [51, 52, 150]. However, stimulation of the cell cycle in *slr-1* mutant background only triggers pericycle cell divisions but fails to initiate LR formation, suggesting SLR/IAA14 mediated coordination of cell cycle activation as well as cell fate respecification are indispensable for successful LR initiation [150]. *slr-1* and *arf7ar19* mutants do not interfere with founder cell identity acquisition but disrupt most early events of LR initiation, including nuclear migration, radial expansion, and asymmetric cell division [5, 21, 51]. Modulating the degradation dynamics of SLR/IAA14 through point mutations demonstrated that IAA14 turnover rate strongly correlates with the pace of LR organogenesis. Slowing its degradation rate leads to delays in LR initiation, implying that SLR/IAA14 functions as an auxin-induced timer for LR initiation [65].

Downstream molecular components redundantly regulated by ARF7 and ARF19 include several auxin-regulated genes. In particular, the expression of several LBD transcription factors, including LBD16, LBD17, LBD18, LBD29 and LBD33 is dependent on the SLR/IAA14-ARF7-ARF19 auxin signalling pathway. ARF7 and ARF19 directly bind to auxin response elements in the promoters of *LBD16* and *LBD29* to activate their expression during LR initiation [110, 111]. Consistently, overexpressing either LBD16 or LBD29 partially rescues the LR defect in *arf7arf19* mutants [111]. Additionally, repressing the transcriptional activity of LBD16 by fusing it to a transcriptional repressor domain (LBD16-SRDX) severely impairs LR initiation and phenocopies several LR defects observed in *slr-1* and *arf7arf19* mutants [56, 57, 111]. Polar nuclear migration of LRFCs is blocked in LBD16-SRDX, but the nuclei can radially expand symmetrically, leading to symmetric anticlinal division of the cells and subsequent arrest in LR formation [56, 57]. LBD18 and LBD33 function as heterodimers to coregulate cell cycle reactivation during LR initiation by stimulating the transcription of E2Fa, a cell cycle regulator [12]. Overexpression of LBD18 can also stimulate LR formation in *arf7arf19* mutant, similar to LBD16 and LBD29. However, single loss-of-function mutants of LBD16, LBD18 and LBD33 only display a moderate reduction in the number of LRs formed, and synergistic reduction is observed in higher order mutants. Their single loss-of-function mutants does not completely preclude LR formation, indicating that LBDs display a high functional redundancy in LR organogenesis [12, 56, 57, 87].

Post-translational modifications, such as ubiquitination and phosphorylation, influence the output of SLR/IAA14-ARF7-ARF19-LBD auxin signalling module. Intracellular auxin is perceived by the F-box receptor proteins TIR1/AFB1–3, which bind to AUX/IAA transcriptional repressors and promote their ubiquitination. This ubiquitination targets AUX/IAAs for degradation by the 26S proteasome, thereby derepressing ARF activity [29, 63, 76]. BRASSINOSTEROID-INSENSITIVE2 (BIN2)-mediated phosphorylation attenuates the interaction of ARF7 and ARF19 with AUX/IAAs, consequently increasing the abundance of free ARFs and enhancing their DNA binding capacity. This results in an auxin-independent increase in the binding of ARF7 and ARF19 to the promoters of *LBD16* and *LBD29* [19]. Consistently, LR density is higher in *bin2*, gain-of-function mutant and reduced in *bin2-3*, loss-of-function mutant [19, 93].

Epigenetic factors, such as histone deacetylases and the chromatin remodelling factor PICKLE (PKL), play a role in SLR/IAA14 mediated inactivation of ARF7 and ARF19. Inhibiting histone deacetylase activity overcomes the LR block in *slr-1* mutant, but not in the *slr-1arf7arf19* triple mutant. Likewise, mutations in PKL *(pkl/ssl2)* also restore LR formation in the *slr-1* mutant background, but not in the *slr-1arf7arf19* triple mutant [53]. Transcriptional repression mediated by PKL negatively regulates LR initiation through repressing *LBD16* promoter activity, thereby restricting formative divisions. PKL forms a repressive complex with RETINOBLASTOMA-RELATED 1 (RBR1) that binds to and represses the activity of *LBD16* promoter in an auxin-dependent manner. This suggests that auxin signalling regulates LBD16 expression via a dual interconnected regulatory mechanism involving the SLR/IAA14-ARF7-ARF19 module and the PKL–RBR1 repressor complex [53, 112]. Another negative regulator of LR formation, SHI-RELATED SEQUENCE5 (SRS5), functions by directly downregulating the expression of LBD16 and LBD29. This inhibitory effect is released by auxin through ARF7 and ARF19 mediated repression of SRS5 [151].

FOUR LIPS (FLP), a direct target of ARF7, works in conjunction with ARF7 itself to directly regulate the transcription of the auxin efflux carrier PIN3 during LR initiation. Mathematical simulations suggest that ARF7-FLP form a feed-forward loop to amplify and sustain PIN3 expression in pericycle cells following the transient auxin stimulus. This proposed configuration could enable an extended response to auxin in LRFCs, allowing them to reach the auxin threshold required for the transition from founder cell identity to LR initiation [18]. In addition to being expressed in pericycle

founder cells, PIN3 is also transiently induced in endodermal cells overlying LRFCs during LR initiation. It displays laterally polarised expression towards the pericycle founder cells, establishing an auxin reflux pathway from overlying endodermal cells to founder cells to reinforce auxin flow. Interfering with this pathway results in delayed transition from founder cells towards LR initiation phase in *pin3* mutant, a phenotype that can be restored by specifically expressing PIN3 in endodermis [97].

As discussed earlier, the coordination between pericycle and endodermal cell layers is crucial for LR initiation. A recent study by Peng et al., [115] has demonstrated that UBIQUITIN-SPECIFIC PROTEASE14, DA3 co-ordinates these responses by modulating auxin signalling in pericycle and endodermis via SHY2, ARF7, and ARF19. In pericycle and endodermal cells, DA3 negatively regulates mRNA and protein expression levels of ARF7 and ARF19, leading to the downregulation of LBD16 expression in the pericycle and suppressing LR initiation. In the endodermis, DA3 enhances its repressive effect on LR initiation by deubiquitinating SHY2 and upregulating SHY2 levels. DA3 and SHY2 have synergistic effect on suppressing LR initiation, they commonly interact with ARF7 and ARF19, thereby regulating LBD16 expression in the pericycle. In line with this, loss of function of DA3 promotes LR initiation but suppresses LR emergence [115].

The patterning of the first asymmetric divisions is regulated by signalling peptides of GOLVEN/root growth factor/CLE-like (GLV/RGF/CLEL) family. Excess GLV6 activity, achieved through either overexpression of GLV6 or treatment of roots with GLV6 peptides, impedes nuclear migration and disrupts the asymmetric pattern, resulting in non-formative symmetric divisions without altering the progression of the cell cycle. Excess GLV6 peptide also induces ectopic anticlinal pericycle divisions through MPK6 phosphorylation in an RGI receptor-dependent process [43, 44, 46]. Furthermore, the precise control of cell division plane orientation during asymmetric formative divisions involves AURORA (AUR) kinases. The *aur1-2aur2-2* double mutant exhibits defects in the positioning of the division plane during LR initiation, although the preceding nuclear migration remains unaffected. This results in aberrantly oriented formative divisions, disrupting the formation of the typical layered structure and interfering with the subsequent development and emergence of LRs [92, 146].

The maintenance of mitotic potential in XPP cells, essential for LR formation, depends on the activity of ABERRANT LATERAL ROOT FORMATION4 (ALF4). In the LR-deficient *alf4-1* mutant, XPP cell divisions during LR initiation are either blocked or delayed before mitosis. This mutant also exhibits a reduced DR5 response and displays resistance to auxin [3, 16, 32, 40]. ALF4 functions as a repressor of Skp1-Cullin1-F-box (SCF) complexes, SCF^{TIR1} and SCF^{SLY1}. Loss of ALF4 function causes defects in ubiquitin–protein conjugation, stabilizing SCF substrates IAA17 and REPRESSOR OF GA1-3 (RGA). This leads to the accumulation of AUX/IAA proteins and DELLA repressors, which could partly contribute to the reduced auxin response and LR defects observed in *alf4* mutants [3].

Auxin regulates additional components of the cell cycle machinery to regulate asymmetric divisions essential for proper tissue patterning. In LRFCs, auxin triggers the G1-to-S phase cell cycle transition by downregulating gene expression and protein accumulation of Inhibitor-Interactor of CDK/Kip Related Protein2 (ICK2/KRP2). ICK2/KRP2 acts as an inhibitor of D-type cyclins (CYCD) 2;1- cyclin-dependent kinase (CDK) A complex activity. Loss-of-function mutants in ICK2/KRP2 display a higher LR density, while ectopic overexpression of KRP2 prevents cell cycle activation for formative divisions, thereby inhibiting LR initiation [67, 130]. ICK2/KRP2 interacts with and mediates the nuclear localization of the CDKA,1–CYCD2;1 complex, resulting in an inactive complex due to the inhibitory effect of ICK2/KRP2. Auxin transiently stimulates the activity of the nuclear reservoir of CDKA;1–CYCD2;1 complex, through the degradation of ICK2/KRP2, subsequently promoting cell division [130]. Other cell cycle regulating factors governing LR initiation include F-box proteins, S-PHASE KINASE-ASSOCIATED PROTEIN (SKP) 2A and SKP2B. Despite their high homology, they exert opposite regulatory effects. SKP2A promotes cell division, whereas SKP2B functions as a negative regulator of cell division in LRFCs [74, 75, 95].

Post-initiation events: LR outgrowth and emergence

Following initiation, the process of LR organogenesis involves coordinated cell division, expansion, and differentiation to generate a dome shaped LRP that eventually acquires a similar organization as the primary root meristem. Simultaneously, the developing LRP traverses three overlying cell layers; the endodermis, cortex and epidermis before emerging at the surface of primary root (Fig. 2). While the final shape and cellular pattern of LRP are stereotypical, the patterns of cell divisions during LRP morphogenesis do not follow a stereotypical order, with the exception of the first asymmetric division during initiation. Following the first anticlinal cell division, subsequent cell divisions are governed by few strict rules and regularly switch in division plane orientation [92, 157].

Table 1 Overview of mutant and transgenic lines affected in LR outgrowth and emergence

Mutants/Transgenic lines	LR phenotype	Stage affected	References
plt3plt5plt7	Loss in tissue/layer identity from stage II & Reduced number of emerged LRs	LRP patterning and organisation	Du and Scheres, [35]
puchi-1	Additional anticlinal and periclinal cell division in the flanking domain, promotes initiation and clustering of primordia, delay in development and emergence	LRP patterning and morphogenesis	Hirota et al. [68],Trinh et al. [145],Bellande et al. [8]
ahp6	Abnormal pericycle cell divisions orientation at stage I and II	LRP patterning and orientation of cell division	Moreira et al. [105]
atx1-1	Compromised cell proliferation, tissue patterning, enlarged flanking cells delay in outgrowth	LRP patterning, morphogenesis and outgrowth	Napsucialy-Mendivil et al. [109]
myb36-5	Increase in stage IV LRPs, decrease in emerged LR density, More cells at the base and flattened LRPs	LRP morphogenesis and outgrowth	Fernández-Marcos et al. [45]
Atmyb93-1,Atmyb93-2	Increase in stage VII and emerged LRPs, Accelerated outgrowth rate	LR outgrowth and progression	Gibbs et al. [55]
35S::MYC-AtMYB93	Increase in stage I-IV LRPs, slow LR developmental progression and decrease in emerged LR density		
tas3a-1	Increase in stage I and II LRPs and shorter LRs	LR outgrowth and progression	Marin et al. [99]
35S::TAS3A	Increase in stage V-VII LRPs and elongated LRs		
35S::5MIR156	Decrease in Stage I-IV LRP and increase in emerged LRs	LR outgrowth and progression	Yu et al. [140]
P35S::rSPL3; PSPL9::rSPL9; PSPL10:rSPL10	Increase in Stage I-IV LRP and decrease in emerged LRs		
tob1-1	Increased numbers of LRP, emerged LRs, and accelerated growth rates	LRP organogenesis	Michniewicz et al. [101]
prh1-1; prh1-2; prh1-3	Increase in Stage I-IV LRP, decrease in Stage V-VIII LRPs and delayed emergence	LR progression and emergence	Zhang et al. [61]
tmk1-1tmk4-1	Disorganised cell division patterning and no emerged LRs	LRP patterning and emergence	Huang et al. [71]
mkk4,5RNAi-est; mkk4mkk5	Disorganised cell division patterning, Increase in Stage I-IV LRPs and decrease in emerged LRs		Huang et al. [71], Zhu et al. [104]
mpk6 – / – mpk3RNAi-est; MPK3SR and MPK6SR	Disorganised cell division patterning, Increase in Stage I-IV LRPs and decrease in emerged LRs		Huang et al. [71], Zhu et al. [104]
ida	Flattened LRPs, Increase in Stage IV-VII LRPs and decrease in emerged LRs	LR morphogenesis and emergence	Kumpf et al. [80]
haehsl2	Flattened LRPs, Increase in Stage IV-VII LRPs and decrease in emerged LRs	LR morphogenesis and emergence	Kumpf et al. [80]
fra2; lue1; ktn1-2	Disorganised cell division patterning and reduced number of emerged LRs	LR morphogenesis and emergence	Ovečka et al., [113]
d35S::PIP2;1	Flattened LRPs, Increase in Stage II-VII LRPs and decrease in emerged LRs	LR morphogenesis and emergence	Péret et al. [117]
pip2;1-1; pip2;1-2	Flattened LRPs, Increase in Stage II-VII LRPs and decrease in emerged LRs	LR morphogenesis and emergence	

Table 1 (continued)

Mutants/Transgenic lines	LR phenotype	Stage affected	References
ore1-2	Reduced frequency of overlying cell death at Stage I-IV and delayed emergence	LR emergence	Escamez et al. [42]
pdlp5-1	Faster LR emergence and increase in emerged LRs	LR emergence	Sager et al. [127]
PLDLP5OE	Delayed LR emergence and reduced number of emerged LRs		
lax3	Increase in Stage I LRP and reduced emergence/decrease in emerged LRs	LR emergence	Swarup et al. [141], Porco et al. [120]
lbd29-1	Increase in Stage I, IV and V LRP and reduced emergence/decrease in emerged LRs	LR emergence	Porco et al. [120]
gLBD29-SRDX	Increase in Stage V LRP and decrease in emerged LRs		
lbd18-1	Reduced number of emerged LRs	LR emergence	Lee et al. [87]
Pro35S:LhGR:EXP17RNAi	Reduced number of emerged LRs	LR emergence	Lee and Kim [88]
35S::EXP17	Increase number of emerged LRs		

Cells within the LRP display a characteristic layered organization, that is used for classification into eight histologically defined developmental stages, each corresponding to the to the typical number of cell layers formed [10, 38, 83, 94]. Three notable landmarks contribute to the functional patterning of LRPs during LR morphogenesis. I) The first round of periclinal divisions during the transition from stage I to stage II establishes a new growth axis and imparts an asymmetric identity to the inner (proximal) and outer (distal) layers (Fig. 2). This transition is marked by the differential expression of transcription factors such as PLT1, PLT2, SHORTROOT, and SCARECROW between the two cell layers, with PLT transcription factors also playing a crucial role in regulating this transition phase [35, 58]. II) The establishment of the de novo meristem and new auxin response maximum in the primordium at the stage IV to V transition, coinciding with the LRP traversing the overlying endodermis. This transition is also associated with the LRP shape change from flat-topped to dome shaped as the biomechanical constraint is relaxed upon breaching the tough Casparian strip barrier of the endodermis (Fig. 2) [58, 83, 92, 154]). III) The transition from stage V to stage VII is characterized by an accelerated LRP outgrowth, primarily driven by basal cell elongation within the primordium. This results in the development of an anatomically recognizable meristematic structure reminiscent of the primary root meristem [58].

In the LRP, two distinct lateral growth domains (central and flanking zones) are specified with different identities, cell growth and division patterns. These domains define the growth boundaries of the LRP. The central domain, characterized by a relatively high auxin concentration, contains rapidly proliferating inner cells. It is flanked by peripheral cells that originate from restricted tangential and radial cell divisions, resulting in anisotropic tissue growth, giving rise to the prominent dome-shaped structure of the primordium [50, 58, 85, 131]. The shape of the LRP is largely conserved, and its morphology is primarily influenced by mechanical properties of the overlying tissues during emergence [92, 154]. LR emergence involves a series of coordinated events between the LRP and the surrounding tissues. During this process, the endodermis accommodates LRP growth by changing shape and undergoing drastic volume loss. The plasma membranes from both sides of the endodermis fuse while maintaining membrane integrity, and the Casparian strip is locally degraded, allowing a confined opening of the network for the passage of the emerging LRP [154]. In contrast, cortical and epidermal cells undergo cell separation facilitated by cell wall remodelling enzymes and regulation of aquaporin-dependent tissue hydraulics to promote LR emergence [80, 117, 118, 120, 141]. Therefore, in addition to primordium organisation and patterning, the coordination of biochemical and biomechanical responses between LRP and the surrounding tissues is paramount for outgrowth and emergence of the LRP. LR outgrowth and emergence is a complex process regulated in Arabidopsis by distinct signalling pathways and genetic regulators at various stages [comprehensively discussed in reviews by [4, 137, 155] and summarised in Table 1].

Conclusion

The work discussed above reveals that LRs, major determinants of root architecture, are formed by means of multiple developmental mechanisms. The ability of the plant to tune LR formation for adaptation and survival depends on its ability to control these mechanisms according to needs. Recent studies have generated significant insights into intrinsic mechanisms regulating LR development at the cellular, organ and structural level. Despite this considerable progress, significant gaps remain in our understanding of the causal connections and crosstalk between different pathways, at the spatiotemporal level and under different growth conditions. In addition, much remains to be learned about how plants integrate endogenous regulatory pathways with environmental cues.

Author contributions

KY, BS and VW wrote the manuscript, BS, JV, VW have edited the manuscript.

Funding

KY and JV were funded by the NWO VIDI grant no. 864.13.008.

Availability of data and materials

No datasets were generated or analysed during the current study.

Declarations

Competing interests

The authors declare no competing interests.

Received: 11 July 2024 Accepted: 17 September 2024
Published online: 02 October 2024

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