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Current Opinion in Plant Biology

Talking through walls: mechanisms of lateral root emergence in *Arabidopsis thaliana*

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Lateral roots are formed postembryonically and determine the final shape of the root system, a determinant of the plants ability to uptake nutrients and water. The lateral root primordia are initiated deep into the main root and to protrude out the primary root they have to grow through three cell layers. Recent findings have revealed that these layers are not merely a passive physical obstacle to the emergence of the lateral root but have an active role in its formation. Here, we review examples of communication between the lateral root primordium and the surrounding tissues, highlighting the importance of auxin-mediated growth coordination as well as cell and tissue mechanics for the morphogenesis of lateral roots.

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Current Opinion in Plant Biology 2015, **23**:31–38

This review comes from a themed issue on **Growth and development**

Edited by **Niko Geldner** and **Sigal Savaldi-Goldstein**

http://dx.doi.org/10.1016/j.pbi.2014.10.005

Introduction

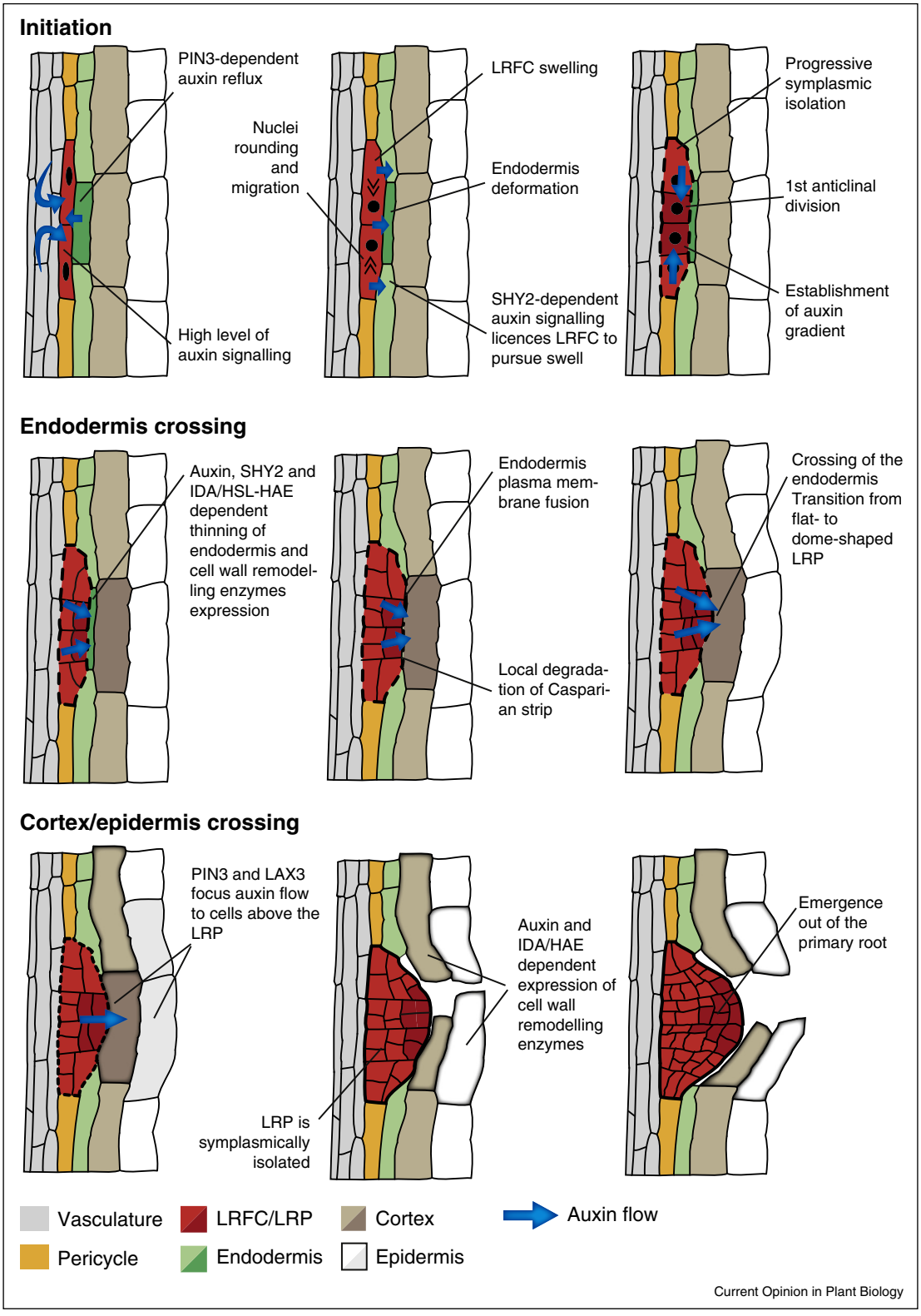
The root system of *Arabidopsis thaliana* consists of an embryo-derived primary root from which secondary lateral roots are continuously produced. From this simple scheme, elaborate root system architectures are shaped, which determine the efficacy of the plant anchorage, water uptake and nutrient acquisition. Lateral root formation entails the specification of founder cells, their coordinated division and differentiation to produce an organ primordium. Lateral root founders derive from cells of the pericycle, an internal tissue surrounding the central vascular cylinder. Lateral root formation commences when these founders divide and create a dome-shaped lateral root primordium (LRP), which has to cross three overlying tissues to emerge at the surface of the parent root: the adjacent endodermis, the cortex and the outermost layer, the epidermis. The rigid cell wall linking plant cells to each other prevents any sliding or migration. To preserve the structural and functional integrity of the primary root, it is necessary to coordinate growth and proliferation within the LRP and the responses of the overlying tissues. Auxin plays a pivotal role in coordinating these responses [1]. LRP development correlates with the establishment of an auxin response maximum at the primordium tip, a process dependent on auxin transport mediated by PIN auxin efflux transporters [2,3]. At the transcriptional level, auxin modulates the expression of different sets of genes by triggering the degradation of the repressors AUX/IAA, which interact with the auxin response factors (ARFs) transcription factors [1]. As the ARFs and the AUX/IAA are members of multi-genes families, the transcriptional effects of auxin depend on its concentration and the combinatorial of expression of AUX/IAA and ARFs [4,5].

Here, we focus on the processes that ensure the coordinated outgrowth of the lateral root from the main root. We discuss chronologically the morphogenesis of lateral roots, starting with its initiation, the crossing through the endodermis, the cortex and epidermis. We highlight the prominent role of intercellular communication for harmonizing the growth of the lateral root primordium and the spatial adaptation of overlying tissues. In particular, we point out the links between auxin, the modification of cell wall properties and the resulting changes in biomechanics. We make the case that mechanical and biochemical forces link intimately during lateral root organogenesis and contribute to its robustness.

Lateral root initiation: formation of a one-layered primordium

The decision to initiate a lateral root is formed in the basal meristem by the specification at regular interval of lateral root founder cells (LRFCs). This process is the result of a complex interplay of oscillating transcription, auxin transport and auxin signaling [6–10]. The mechanisms by which LRFC are specified and regularly spaced along the primary root have been reviewed recently [11] and will not be covered. In *Arabidopsis*, lateral roots are initiated when single or pairs of pericycle cells, always facing the xylem poles, undergo asymmetric anticlinal divisions, creating a single layered primordium containing up to ten small cells [12–15]. This single layered primordium typically consists of small group of central cells flanked by longer cells. Prior to initiation, auxin responsiveness increase in the LRFCs [16] (see Figure 1) and perturbation of polar auxin transport is sufficient to block initiation [17]. Two different AUX/IAA-ARFs modules,

Figure 1



Phases of lateral root formation in *Arabidopsis thaliana*. For each phase (initiation, crossing of the endodermis and of the cortex/epidermis), the main events are indicated. Tissues are color coded, darker shade indicates high level of auxin signaling.

one involving IAA14-ARF7/ARF19 [18–21] and one IAA12-ARF5 [22], control the reactivation of the cell cycle in the LRFC and the acquisition of diverging identities by the central and peripheral cells.

As in other developmental contexts [23], cytokinins antagonize the effects of auxin on lateral root initiation. By altering the amount of cytokinins, their perception or signaling, lateral root initiation is inhibited [24–26]. Cytokinins repress the expression of several auxin efflux carriers of the PIN family [26], trigger the rapid degradation of existing PIN1 [27] and its polar localization [28], which perturb auxin flow and therefore inhibit the ability of LRFC to enter mitosis [29,30].

Prior to the first division marking lateral root initiation, the nuclei of LRFCs round up and, in case of abutting pair of LRFC, migrate toward the cell wall common to the two cells [8]. Recently, Vermeer *et al.* [31••] have used light sheet microscopy [32] to observe that concomitantly to the rounding and migration of their nuclei, the LRFCs increase in volume and the overlying endodermis shrinks and deforms [31••] (see Figure 1). This swelling of the pericycle is mimicked by treatment with auxin and is dependent on an early auxin perception in the endodermis. The AUX/IAA auxin repressor SHY2, expressed in the endodermal cells directly overlying the LRP, controls there the responses of the endodermis to auxin [33]. Plants expressing a SHY2 loss-of-function allele (*shy2-24*) that enhances endodermal auxin responsiveness show accelerated lateral root emergence, compared with wild-type. Conversely, plants expressing the gain-of-function allele *shy2-2* that reduces auxin responsiveness in the endodermal cells show delayed lateral root emergence [33]. By expressing *shy2-2* only in the endodermis, Vermeer *et al.* could block the swelling of the LRFC and the execution of the first division [31••]. It therefore appears that auxin accumulation in the LRFC is perceived in the endodermis by a SHY2-containing module; the endodermis signals back to the LRFC by an unknown factor and licenses the LRFCs to pursue swelling and to divide. This interplay between biochemical and mechanical regulation defines a checkpoint for lateral root initiation. The change in the mechanical constraint induced by a change in pericycle cell geometry relates lateral root initiation to the observation that root bending is a potent inducer of lateral root formation [34–36]. The mechanical deformation of cells induced by bending would modify auxin flow and therefore concentration in the pericycle and the adjacent vasculature [34,35] and trigger lateral root initiation.

Perception of pericycle-derived auxin in the endodermis also induces auxin reflux from the endodermis into the pericycle [37•]. Shortly after LRFC specification, the expression of the auxin-efflux carrier PIN3 is induced in a small group of endodermal cells adjacent to the lateral root primordium. In these cells, PIN3 is localized in the membrane facing the LRP. The *pin3* mutant shows a delay in lateral root initiation, a phenotype rescued by the endodermis-specific expression of PIN3-GFP. The endodermal PIN3-driven reflux of auxin to the primordium therefore participates in the induction of the second burst of auxin, necessary for the proper division of the LRP [37•] (see Figure 1).

Plant cells are interconnected by plasmodesmata (PD), membrane-lined channels that traverse the cell walls of neighboring cells and allow symplasmic movement of molecules between cells [38,39]. The turn-over of callose (b-1,3-glucan) regulates the degree of PD opening, which plays a key role in many developmental processes [40]. A recent report has shown how PD-mediated connectivity plays a role during lateral root formation. Using a phloem-expressed free GFP as a tracer for symplasmic domains, Benitez-Alfonso *et al.* [41••] observed that the connectivity between the LRP and the surrounding tissue gradually decreases. By the time the primordium forms a four-layered structure, it is completely isolated (see Figure 1). This dynamic regulation of symplasmic connectivity is accompanied by differential callose accumulation at the PD [41••]. The two PD-localised glucanases PdBG1 and PdBG2 are expressed in the LRFCs and their expression persists after lateral root initiation. The authors observed higher levels of callose and clusters of LRP primordia in the *pdbg1/pdbg2* double mutant, demonstrating that reduced degradation of callose has an impact on lateral root formation. A precise control of the symplasmic connectivity at early stages of LRP development restricts priming and initiation by regulating the movement of unknown factors impeding lateral root initiation. The nature of these factors remain elusive, but the phenotypes observed by Benitez-Alfonso *et al.* [41••], are reminiscent of the ones produced by mutation of the receptor-like kinase ARABIDOPSIS CRINKLY4 (ACR4). ACR4 is specifically expressed in the small cells located at the center of the single-layered primordium and its mutation leads to clusters of small pericycle cells indicating ACR4 represses additional asymmetric anticlinal divisions in the cells flanking LRFCs. ACR4 is also expressed in the columella and the epidermis/lateral root cap initials of the root apical meristem, where it also restricts divisions in the neighboring cells [42]. In these cells, ACR4 preferentially accumulates at the plasmodesmata [43], opening the possibility ACR4 may, in the LRFCs, be involved in the intercellular traffic of a factor repressing formative division at the periphery of the lateral root primordium.

Crossing the endodermis

On its way to the surface, the first layer the lateral root primordium crosses is the endodermis. The endodermis is a tissue, which in its structure and its function resembles the polarized epithelia of metazoans [44]. Endodermal cells are surrounded by an hydrophobic lignin-rich structure called

the Casparian strip [45,46] that sets up a diffusion barrier between the extracellular space of the root cortex, connected to the soil, and the vascular tissue, connecting all plant organs. By its chemical nature, an inelastic polymer of phenol, the Casparian strip is resistant to chemical degradation and rigid. How the endodermis is remodeled during the growth and emergence of a LRP has only been solved recently [31••].

Vermeer *et al.* [31••] observed that endodermis cells suffer a dramatic change of shape upon growth of the LRP. The cells become progressively thinner, lose volume, up to the point where opposing plasma membranes fuse (see Figure 1). Nonetheless, vacuole and plasma membrane integrity is never compromised. The endodermis cells remain attached to each other and the Casparian strip is locally degraded to allow for a confined opening of the lignin network where the primordium penetrates. This cellular accommodation of the endodermis differs from the response of the cortex and endodermis layers that separate from each other to make way for the emerging LRP (see below). The localized break down of the Casparian strip above the tip of the LRP ensures that on the flanks of the LRP no gaps are created in the diffusion barrier the endodermis provides, preserving the isolation of the vascular bundles from the outside [44]. The remodeling of part of the Casparian strip, additionally suggests mechanical constraints may play a role in the morphogenesis of the LRP itself. This is supported by two observations. First, the transition from a 'flat-topped' into a 'dome-shape' primordium occurs upon endodermis crossing [47••] (see Figure 1). Second, the tangential and oblique planes of division that break the bilateral symmetry of the LRP and are associated with its radialisation, are observed when the LRP crosses the endodermis [47••]. What is the trigger of the thinning of the endodermis upon growth of the LRP? In plants expressing the stabilized *shy2-2* form selectively in the endodermis, LRFCs activation is blocked and no lateral roots form [31••]. This block is overturned by auxin treatment. Primordia of auxin-treated plants expressing *shy2-2* in the endodermis were flattened and the endodermis stayed turgid [31••]. Therefore, endodermis accommodation to the growth of the LRP involves, a minima, a cell autonomous SHY2-dependent auxin perception in the endodermis. The change in the morphology of the LRP could result from the mechanical resistance of the endodermis. This imbalance in force, is reminiscent of the phenotype observed when the ability of the overlying tissue to yield to the primordia is compromised [47••] or the water movements associated with turgor pressure are perturbed [48••]. Expression of a dominant negative version of the AUX/IAA AXR3 in the three layers above the LRP, leads to delayed emergence and a flattening of the primordia, similarly to the misexpression of the PIP2;1 aquaporin [48••] (see below).

The drastic changes in endodermis cells shape induced by auxin entail modifications in the properties of the cell wall. Kumpf *et al.* recently uncover a link between auxin-mediated thinning of the endodermis, cell wall properties and signaling by receptor-like kinases [49••]. The signaling peptide INFLORESCENCE DEFICIENT IN ABSCISSION (IDA), and its receptors, the leucine-rich repeat-like kinases HAESA (HAE) and HAESA-like2 (HSL2), form a signaling module involved in the regulation of cell wall remodeling enzymes during cell separation and abscission in flowers [50]. All elements of this module are also expressed in the endodermis, cortex and epidermis cells overlying the developing LRP. In plants with mutations in *IDA*, *HAE*, or *HSL2*, the LRP show delays in crossing the endodermis, which fail to thin [49••], implicating this peptide/receptor signaling module in the passage of the LRP through the endodermis. The expression of IDA, HAE and HSL2 is induced by auxin and all three are required for the proper expression of the cell wall remodeling enzymes XTH23/XYLOGLUCAN ENDOTRANSGLYCOSYLASE6 (XTR6, [33]) and the expansin EXP17 [51] in the endodermal cells above the LRP. These results suggest a model in which auxin accumulation in the endodermis above the LRP induces IDA expression, which signals through HAE and HSL2 receptors to up-regulate the expression of cell wall remodeling enzymes genes like XTR6 and EXP17 controlling endodermis thinning.

Crossing the cortex and epidermis

Once it has crossed the endodermis, the lateral root must pass the cortex and epidermis layers to emerge at the surface of the primary root. Scanning electron micrographs of LRP emerging across the epidermis and cortex show cells barely changing shapes but pushed apart by the emerging lateral root [49••,51] (see Figure 1). Separation of these cells requires the degradation of the pectin-rich middle lamella that joins adjacent cells together.

Pectate lyases (PL) degrade pectins once those are demethylesterified, a reaction catalyzed by pectin methyl esterases (PME). This combined requirement for PME and PL activities to degrade the lamella prevents the cell wall loosening of the LRP cells in contact with overlying tissues. Staining for methylesterified pectin reveal that the cell walls of the LRP are more methylesterified than the ones of the overlying tissue, which could restrict PL activity to these tissues [51]. Transcript profiling of plants treated with auxin reveals the upregulation of several cell wall remodeling enzymes, including the pectin methyl esterase (AtPME1), the alpha-expansin AtEXP1 and the pectin lyases AtPLA1 and AtPLA2, when LRPs are about to emerge or already emerged [51], suggesting their involvement in the separation of cortex and epidermis cells upon emergence. Pectins are also degraded by polygalacturonase (PG). Auxin and the IDA-HAE signaling module activate the expression of PG ABSCISSION

ZONE ARABIDOPSIS THALIANA (PGAZAT) [52], which is detected in the cortical and epidermal cells surrounding the emerging LRP [49••].

The spatial expression and activity of these cell wall remodeling enzymes has to be tightly regulated to preserve the integrity of the main root. Canalization of the auxin flow in the LRP and in the cortex and epidermis cells directly overlying it is instrumental [2,16,33]. The auxin transporters LAX3 and PIN3 contribute to the focusing of the auxin flux to the cortex and epidermal cells over the LRP and the local auxin-dependent induction of cell wall remodeling enzymes [33,53•]. LAX3 is an auxin uptake transporter that mediates auxin accumulation in the cortical and epidermal cells where it is expressed [33]. Interestingly, LAX3 expression is observed early on during the LRP formation process, before any changes in the morphology of the new LRP and overlaying tissues [53•]. Auxin accumulates at the tip of the growing LRP [2,16] and further enhances the expression of LAX3 in the cortex and epidermis cells directly above [33]; in turn, LAX3 upregulates the expression of the cell wall remodeling enzymes AIR3 (a subtilisin-like protease), XTR6 and AtPLA2 [33]. The cortex and epidermis above the LRP therefore starts softening early on, creating an adequate environment for LRP growth and emergence. To avoid a general softening of all cells in that area, which would render the root susceptible to pathogen attacks, the coordinated expression of LAX3 and PIN3 allows only a small group of cells overlying the primordium to undergo softening. The auxin efflux carrier, PIN3 localizes preferentially at lateral, distal, shootward and rootward faces of cortical cells, mediating auxin movement toward outer tissues [53•]. With an elegant combination of mathematical modeling and experimental testing, Peret *et al.* [53•] showed that the robust expression of LAX3 in two abutting cell files over the LRP entails the sequential activation of PIN3 followed by LAX3. This combination ensures that auxin derived from the LRP and moving toward the outer tissues, turns on LAX3 in exactly two cortex cells. An additional conclusion from this work is the importance of tissue geometry in the process of lateral root emergence: the number of neighboring cells making physical contact and the contact surface between these cells, influences the number of epidermal and cortical cells in which auxin will be transported from the LRP and therefore determines how these cells will cooperate with the primordium during emergence [53•].

As any other plant tissue, growth of the LRP results from relaxation of the cell walls and the extension of the cell driven by its turgor pressure. The turgor pressure is maintained by inflow of water into the cell and a tight control of water transport in the LRP is crucial for its growth. The LRP being symplasmically isolated from the vasculature of the main root [41••], influx of water into the LRP must be through the plasma membrane. Aquaporins are membrane channels that facilitate water movement across cell membranes. Movement of water across the plasma membrane is mediated by members of the PLASMA MEMBRANE INTRINSIC PROTEIN (PIP) family [54]. In their work, Peret *et al.* [48••], demonstrate that auxin regulates water exchange between the stele, the LRP and the overlying tissues by controlling the expression of aquaporins. During emergence, one of the most highly expressed aquaporins genes, PIP2;1, shows an auxin-dependent reduction in expression in cortical cells. In contrast, PIP2;8, is activated at the base of the LRP and the underlying stele. Combining mathematical modeling and experimental approaches, they propose water uptake is repressed in the overlying tissues while water transport from the overlying tissues into the primordium is promoted. The opposite effects of auxin on the two aquaporins PIP2;1 and PIP2;8 illustrates the importance of a precise spatial and temporal tuning of water flow for proper emergence.

Concluding remarks and outstanding questions

Intercellular communication and coordination of responses between cells and tissues are paramount for lateral root formation. A complex interwinding of biomechanical and biochemical interactions between the LRP and the surrounding tissues governs all steps of its formation, from initiation and growth to emergence. Auxin plays a pivotal role for the structural and functional patterning of the LRP; its concentration gradient from the base to the tip is required for the proper organization of the LRP, providing cells with positional cues [2]. In addition, auxin affects the mechanical properties of tissues by focusing the action of enzymes modifying cell wall properties. It also influences the turgor through aquaporin-mediated water supply, promoting the emergence of the LRP through overlaying tissues. Despite tremendous recent progress, many questions remain unanswered. The initial swelling of the LRFC is an important event during initiation that induces a mechanical stress to the overlying endodermis. The origin and the nature of the force driving this swelling remain unknown, as well as the factor signaling back from the endodermis, which allow the LRFCs to pursue their development. We suggest a signal of mechanical origin. Despite staggering evidence, the concrete mechanism by which auxin gradients actually govern cell identity and behavior of individual cells is still poorly understood. The importance of mechanics may go beyond the sole growth of the primordium out of the primary root. In contrast, mechanical constraints may play a more active role in LRP patterning. The passage of the LRP through the endodermis corresponds to the time window during which the lateral root primordium, composed of 3–5 layers, self-sustains and develops a normal lateral root even after excision from the parental root [14]. Since no

discrete meristems are formed in calli [55] and two-layered primordia are unable to form an autonomous meristem [14], there is a developmental transitions between stage 3 and 5 that leads to the acquisition of autonomy. The trigger for the formation of the meristem could be the rupture of the endodermis [47••]. One could speculate that the mechanical and/or biochemical cues are modified upon passage through this rigid cell layer and trigger rearrangements in the LRP, that lead to the formation of an autonomous meristem. There are numerous examples of auxin-driven cell wall modifications of overlying cells necessary for LRP emergence. However, the role of cell wall remodeling during LRP formation may be even more pervasive. Recently, Roycewicz *et al.* [56•] showed individual mutations in a range of enzymes catalyzing cell wall modifications all promoted lateral root formation by speeding up lateral root emergence. This observation leads to the hypothesis that cell wall properties of overlying cells could rather play a regulatory role of LRP development than merely pose a physical constraint. Thus, modification of the cell wall properties and the associated changes in cell geometry could have an inductive role for lateral root morphogenesis. One cannot help but draw parallels to the situation in the shoot apical meristem, where modification of the cell wall properties is sufficient to induce organogenesis [57,58]. There, feedback between biochemical and mechanical forces contribute to the organogenesis of the aerial lateral organs. Cell wall remodeling induced by auxin, alters the mechanical properties of cells, which in turn modifies the flow of auxin [59]. The robustness of plant post-embryonic development may find its source in the complex feedback between biochemical and mechanical cues occurring during organ morphogenesis.

Acknowledgements

We thank S. Wolf for his critical reading of the manuscript. The work in the Maizel laboratory is supported by grants from the Land Baden-Württemberg, the Schaller Stiftung and the CellNetworks cluster of excellence.

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- • of special interest
- •• of outstanding interest

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The authors report the singular spatial accommodation of the endodermis to the growth of the emerging LRP. They show that as the lateral root grows, the endodermis progressively loses volume and the plasma membrane fuses, to let it pass through. They demonstrate that this process, as well as the initial swelling of pericycle cells previous to their first asymmetric division, is dependent on auxin perception in the endodermis.

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