Review

Arabidopsis lateral root development: an emerging story

Benjamin Péret[1*], Bert De Rybel[2,3*], Ilda Casimiro[4], Eva Benková[2,3], Ranjan Swarup[1], Laurent Laplaze[5], Tom Beeckman[2,3] and Malcolm J. Bennett[1]

[1] Plant Sciences Division and Centre for Plant Integrative Biology, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, UK
[2] Department of Plant Systems Biology, VIB (the Flanders Institute for Biotechnology), Technologiepark 927, B-9052 Gent, Belgium
[3] Department of Plant Biotechnology and Genetics, Ghent University, Technologiepark 927, B-9052 Gent, Belgium
[4] Departamento de Anatomia, Biologia Celular y Zoologia. University of Extremadura, E-06071 Badajoz, Spain
[5] Institut de Recherche pour le Développement, UMR DIA-PC (Agro.M/INRA/IRD/UM2), Equipe Rhizogenèse, 911 Avenue Agropolis, F-34394 Montpellier cedex 5, France

Lateral root formation is a major determinant of root systems architecture. The degree of root branching impacts the efficiency of water uptake, acquisition of nutrients and anchorage by plants. Understanding the regulation of lateral root development is therefore of vital agronomic importance. The molecular and cellular basis of lateral root formation has been most extensively studied in the plant model *Arabidopsis thaliana (Arabidopsis)*. Significant progress has recently been made in identifying many new *Arabidopsis* genes that regulate lateral root initiation, patterning and emergence processes. We review how these studies have revealed that the plant hormone auxin represents a common signal that integrates these distinct yet interconnected developmental processes.

Lateral root development

Higher plants exhibit an amazing diversity of root architectures at both the system and anatomical levels [1]. Many dicotyledonous plants, such as *Arabidopsis*, have a primary root that repeatedly branches to generate several orders of lateral roots, whereas the root system of cereal crops, such as rice (*Oryza sativa*) and maize (*Zea mays*), are predominantly composed of adventitious roots [2,3]. Recent genetic studies have identified many genes that are common to lateral, crown and adventitious root development [4]. Although impressive progress has recently been made in crops, *Arabidopsis* remains the best characterized experimental system for studying lateral root development and represents the focus for this review.

Arabidopsis lateral roots originate exclusively from pericycle founder cells located opposite xylem poles [5] (Figure 1). Lateral roots are initiated when either individual [6] or pairs of pericycle founder cells undergo several rounds of anticlinal divisions [6–8] to create a single layered primordia composed of up to ten small cells of equal length (termed stage I; Figure 1). Next, the cells divide periclinally, forming an inner and an outer layer (termed stage II; Figure 1). Further anticlinal and periclinal divisions create a dome-shaped primordium (spanning stages III–VII) that eventually emerges (at stage VIII) from the parental root [7,8] (Figure 1).

In this review, we discuss *Arabidopsis* lateral root development chronologically, starting from the initial

Glossary

Recent advances in the study of lateral root formation have led to the identification of new important regulatory processes. In order to avoid any terminology confusion, we propose here a list of definitions describing each of these processes in chronological order.

Xylem pole specification: the differentiation between the phloem pole and xylem pole pericycle cells giving rise to the diarch symmetry of the *Arabidopsis* root. This distinction between the two cell populations is apparent in the pericycle initial cells as early as the heart stage embryo [15].

Basal meristem: zone situated between the apical meristem and the elongation zone of the root characterized by both cell division and cell elongation processes. The basal meristem has previously been named 'post-meristematic isodiametric growth zone' or 'distal elongation zone' [89].

Priming: xylem pole pericycle cells undergo a cyclic pre-initiation event that is auxin dependent, occurs in the basal meristem and marks the pericycle cells every 15 hours. These primed cells gain pericycle founder cell identity and will become founder cells upon auxin activation higher up in the parental root [21]. This founder cell identity specification can also occur outside the basal meristem by auxin induction [23].

Pericycle founder cells: primed xylem pole pericycle cells that undergo initiation by auxin activation, causing them to become pericycle founder cells and undergo the first anticlinal and asymmetric division.

Pre-initiation events: all events occurring before the initiation process and leading to pericycle cell specification and priming.

Lateral root initiation: the start of the auxin-induced signalling processes in two adjacent primed pericycle cells, including the first anticlinal and asymmetrical division of two adjacent pericycle founder cells. This division creates two small daughter cells that will continue to divide and create the primordium flanked by two larger cells.

Lateral root patterning: the tightly controlled sequence of divisions giving rise to a dome-shaped structure that will eventually give birth to a new meristem.

Lateral root emergence: emergence is the continuous process by which the primordium actively makes its way through the outer tissues out to the rhizosphere. It includes the auxin-induced activation of cell wall remodelling genes facilitating the emergence process [67].

Lateral root formation: the developmental process as a whole that combines all steps from pre-initiation events up to initiation, emergence and meristem activation of the lateral root primordium.

Corresponding author: Péret, B. (malcolm.bennett@nottingham.ac.uk)
* These authors contributed equally to this work..

1360-1385/$ – see front matter doi:10.1016/j.tplants.2009.05.002 Available online 24 June 2009

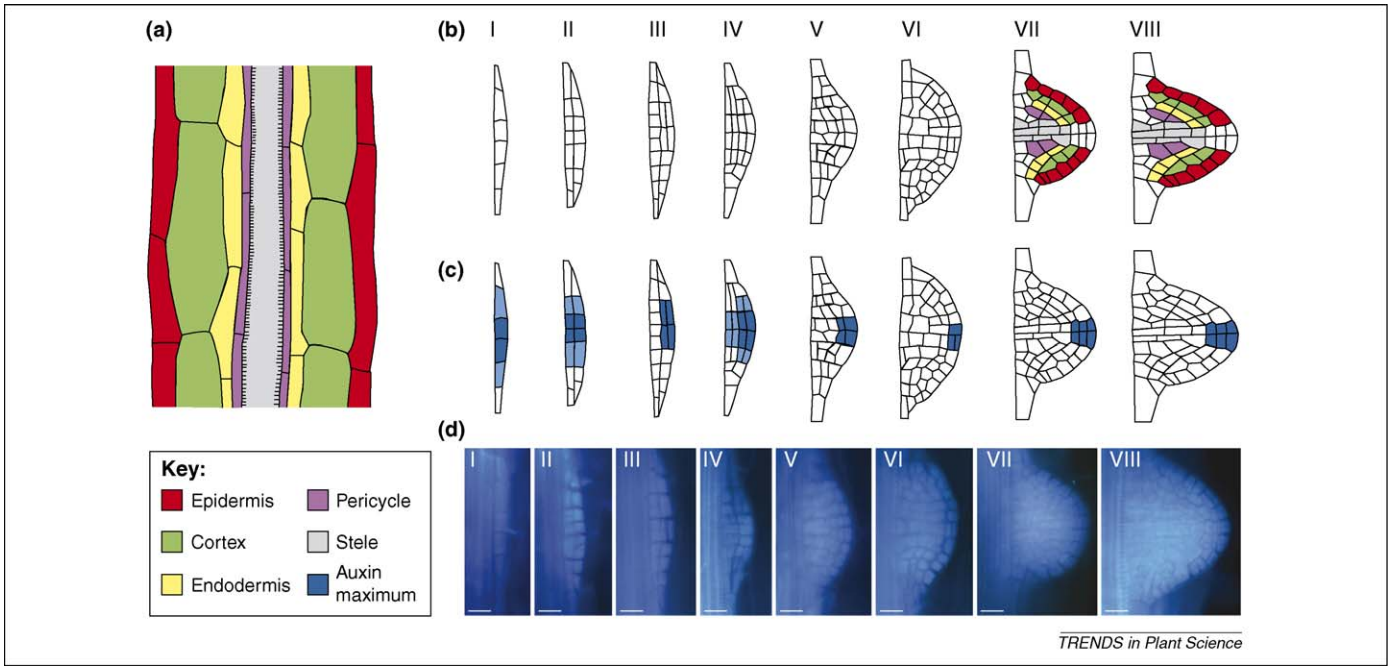

Figure 1. Morphological changes during lateral root development. Lateral roots originate deep within the primary root from the pericycle cells **(a)**. The eight stages of primordium development (roman numbers [6]) are shown **(b)** as well as establishment of the auxin signalling maximum as demonstrated with the *DR5:GUS* reporter (blue gradient **(c)** [30]). The cartoons were drawn from aniline-blue-stained roots for each stage of lateral root development **(d)**. The scale bars represent 20 μm.

specification of pericycle cells opposite the xylem pole, then covering lateral root initiation, patterning and emergence, followed by activation of the new meristem and elongation of the new lateral root (see Glossary). We focus on recent advances in our understanding of the molecular and cellular processes during lateral root development, highlighting new insights into both pre- and post-initiation events, including the previously overlooked issue of how a new organ can emerge through overlaying tissues. Given the space limitations of this article, readers might be interested in several excellent reviews that focus in more detail on specific aspects of lateral root formation, such as auxin-regulated initiation and cell cycle events [9,10], differences between species [4,11] or regulation by nutrients [12–14].

Pre-initiation events: preparing xylem pole pericycle cells to divide

The pericycle represents a heterogeneous tissue consisting of quiescent cells at the phloem poles and cells competent to form lateral root primordia (LRP) opposite each xylem pole [15]. Specification of xylem pole pericycle cell fate (see Glossary) is controlled by the same genetic pathway determining diarch patterning of the underlying root vascular tissues [15,16]. Mutations, such as *lonesome highway* (*lhw*), that disrupt the diarch patterning of xylem poles also block the specification of the overlaying pericycle cells and their ability to form LRP [15]. Although three xylem pole pericycle cell files are dividing during lateral root formation, cell lineage experiments have revealed that only the middle cell file contributes significantly to the establishment of the LRP [17].

After leaving the root apical meristem, pericycle cells at the phloem poles remain in the G1 phase, whereas those at the xylem poles advance to the G2 phase of the cell cycle [18]. This sub-population of pericycle cells at the xylem pole can be considered an 'extended meristem' [19] because, unlike cells at the phloem pole or in other root tissues, they maintain their ability to divide after exiting the root apical meristem. The nuclear protein ALF4 (ABERRANT LATERAL ROOT FORMATION4) is required to maintain xylem pole pericycle cells in a mitosis-competent state because the *alf4* mutation blocks the induction of the mitotic marker *cyclin B1* (*CycB1);1::GUS* but causes overexpression of the earlier cell cycle marker *cyclin dependent kinase B* (*CDKB);1:GUS* in this tissue [20] (Figure 2).

Pericycle cell division in *Arabidopsis* is first detected several millimetres distal to the primary root meristem [7]. However, it has recently been reported that the decision by xylem pole pericycle cells to form a LRP is taken in a zone termed the basal meristem, which is immediately behind the primary root apical meristem [21] (Figure 2). The authors demonstrated that xylem pole pericycle cells in the basal meristem are primed to divide by auxin signalling. Disrupting the auxin response of xylem pole pericycle cells (except those in the apical and basal root meristem zones; Figure 2) blocked the initial asymmetric division of pairs of founder cells [21] (Figure 3). However, their nuclei remained able to migrate to the common cell wall [21] (Figure 3), implying that these pericycle founder cells must have already been exposed to an inductive auxin signal. Hence, priming of pairs of xylem pole pericycle cells to become founder cells by auxin signalling normally occurs close to the root apex in the basal meristem (Figure 2).

Auxin and mechanical signals regulate spacing of pericycle founder cells

Every xylem pole pericycle cell has the ability to divide in response to elevated auxin levels [22,23]. However, only a limited number of xylem pole pericycle cells become

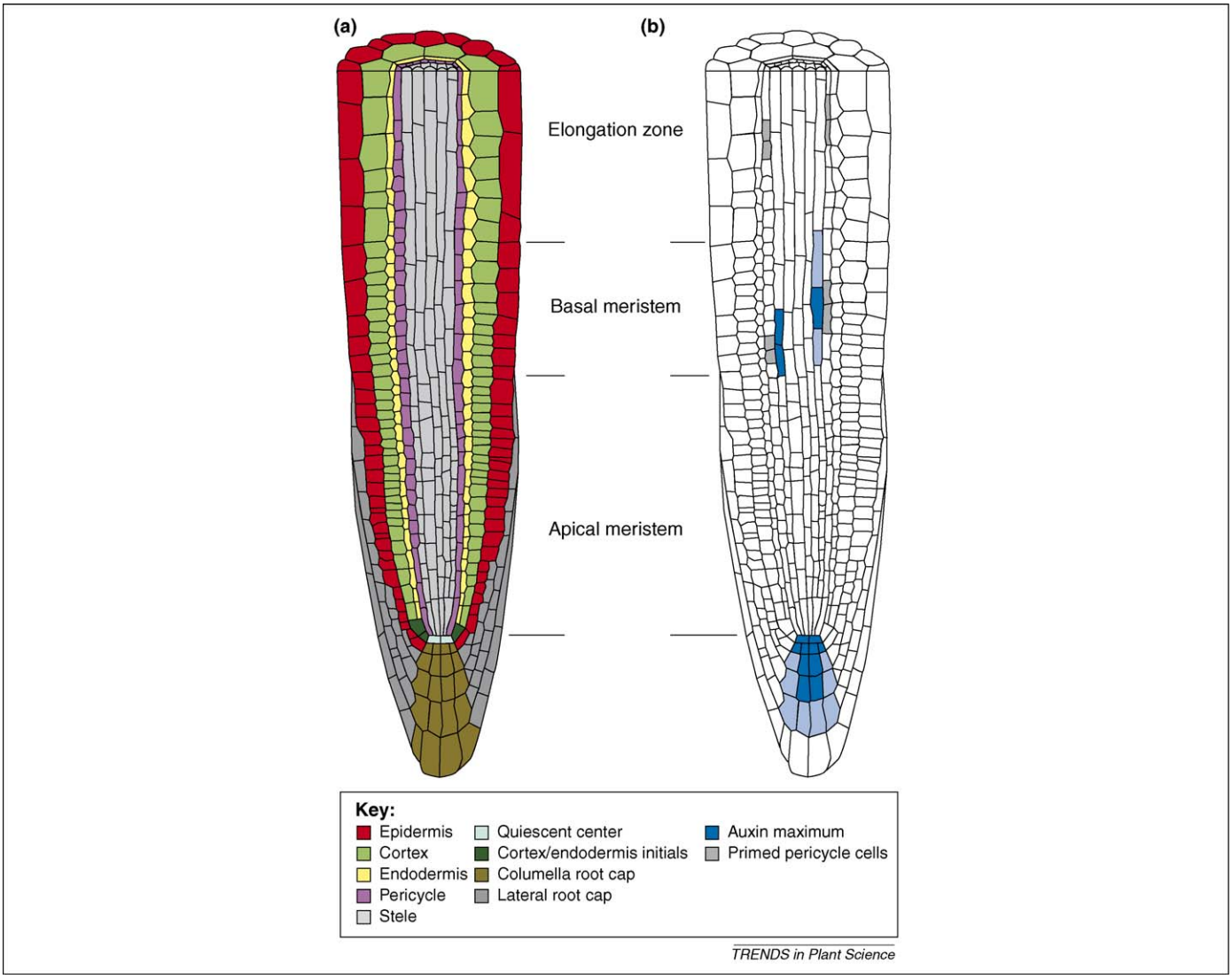

Figure 2. Lateral root priming occurs in the basal meristem. Schematic overview of the different root tissues **(a)** and the auxin signalling maximum present **(b)** as reported by the *DR5::GUS* marker line [21]. The regularly spaced auxin accumulation sites in the protoxylem primes the adjacent pericycle cells to become pericycle founder cells.

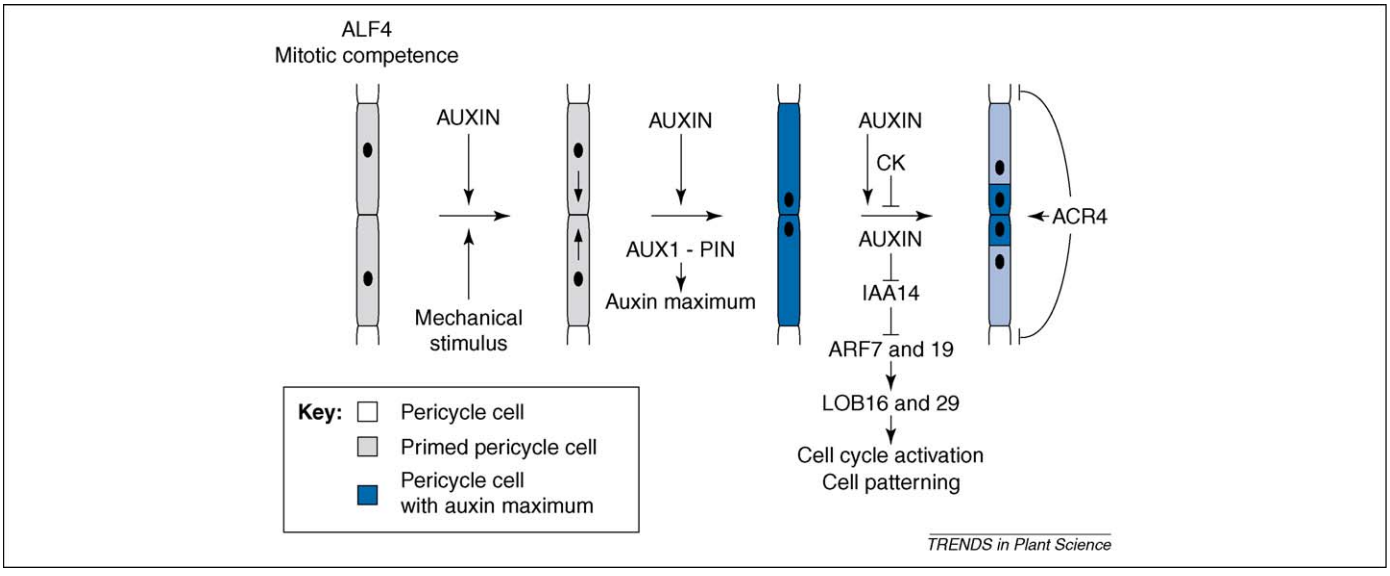

Figure 3. Early events during lateral root initiation. Local auxin accumulation in primed xylem pole pericycle cells activates the auxin signalling cascade. Auxin causes the degradation of IAA14, thereby de-repressing *ARF7* and *19* and activating downstream gene expression. The receptor-like kinase ACR4 promotes formative divisions in the primordium and represses cell divisions in surrounding pericycle cells [54]. Abbreviation: LOB, LATERAL ORGAN BOUNDARIES.

founder cells under normal conditions [8]. Intriguingly, lateral root initiation occurs at a regular rhythm during root growth [24]. Until very recently, the mechanism(s) that patterns the regular spacing of LRP along the root has proved elusive. It has been reported that priming of pericycle founder cells is correlated with an oscillating auxin response (visualized by the DR5::GUS marker) in adjacent xylem cells [21] (Figure 2). The authors demonstrated that these oscillations occurred at regular intervals of ~15 h and were associated with the induction of new LRP in adjacent pericycle cells [21] (Figure 2). *Arabidopsis* roots therefore seem to have an auxin-based oscillatory mechanism that causes the periodic initiation of LRP along the primary root.

The spacing of lateral roots along the *Arabidopsis* primary root can also be influenced by tropic responses and mechanical stimuli [21,25–27]. For example, it has been reported that a gravitropic stimulus can induce LRP to form on the outer side of bending roots [25]. Intuitively, it is hard to reason how higher auxin levels might accumulate in the pericycle on the outside of a root bend when a gravity stimulus diverts the majority of auxin to the lower side of the root [28,29]. Nevertheless, confocal imaging of transgenic roots expressing a *DR5::venusYFP* (*yellow fluorescent protein*) reporter revealed dynamic changes in auxin-responsive gene expression consistent with such a redistribution of auxin between root tissues [26,27]. Via *in silico* modelling, it was hypothesized that higher auxin levels are created in the pericycle on the outside of the bend by mechanically influenced changes in cell sizes. The higher auxin levels trigger expression of the auxin influx carrier AUX1 (AUXIN RESISTANT1), resulting in the creation of an auxin maximum before *de novo* organ formation [26]. By contrast, it was proposed that mechanically induced changes in auxin-responsive gene expression at the site of lateral root initiation required the relocalization of the auxin efflux transporter PIN1 (PIN-FORMED1) in an adjacent protoxylem cell [27]. However, the mechanical induction of LRP cannot be explained by either a PIN1- or AUX1-based mechanism. First, the *pin1* mutant does not exhibit a lateral root defect [30]. Second, lateral root initiation defects in auxin mutants, including *aux1*, can be rescued by a mechanical stimulus [27]. This suggests that a mechanical signalling mechanism must exist independent of auxin to prime pericycle founder cells. Hence, at least two mechanisms seem to be operating in roots to influence positioning of LRP: a mechanical induction pathway and an auxin-based oscillatory mechanism. This makes perfect sense for a root because it enables the spacing of LRP to be responsive to both environmental and endogenous signals.

Although the molecular basis of the mechanical signalling pathway remains to be elucidated, many of the transduction components that mediate auxin-regulated lateral root initiation have recently been identified and characterized [10]. One of the most important auxin signalling proteins regulating lateral root initiation is IAA14 (INDOLE ACETIC ACID14)/SLR1 (SOLITARYROOT1) [31]. IAA14/SLR1 belongs to a family of labile Aux/IAA proteins that function to repress auxin-regulated transcription [31]. A key step in the lateral root initiation pathway is the auxin-dependent degradation of Aux/IAA proteins like IAA14/SLR1. Selected amino acid substitutions in the *IAA14* coding sequence have been reported to stabilize the repressor protein [31]. The resulting gain-of-function *slr-1* mutant does not form LRP as a result of pericycle founder cells failing to undergo formative divisions [31]. Nevertheless, the loss-of-function *iaa14-1* mutant has no obvious phenotype [32], suggesting that other members of the 29-strong *Aux/IAA* gene family might also be involved. Although the gain-of-function mutants *iaa1/axr5* (*auxin resistant 5*) [33], *iaa3/shy2* (*short hypocotyl 2*) [34], *iaa18/crane* [35], *iaa19/msg2* (*massugu2*) [36] and *iaa28-1* [37] are all impaired in lateral root development (Table 1), none block lateral root initiation completely, suggesting further redundancy in the Aux/IAA gene family. This might be due to their differential expression patterns or distinct functionality between these proteins [38].

In the presence of auxin, the F-box proteins TIR1 (TRANSPORT INHIBITOR RESPONSE1) or AFB1–3 (AUXIN RECEPTOR F-BOX PROTEIN1–3) bind to Aux/IAA proteins [39,40], which are then degraded after ubiquitin-dependent targeting to the proteasome [41,42]. TIR1/AFB proteins seem to function redundantly during lateral root development. Whereas single *tir1* and *afb* mutants have either a small or no difference in numbers of LRP, the *tir1 afb2 afb3* triple mutant exhibits a 90% reduction [43,44]. However, it has recently been reported that the *tir1* mutation blocks the doubling in lateral root number observed in wild-type seedlings grown in phosphate (Pi)-poor media [44]. TIR1 expression in the pericycle is induced by low Pi availability, whereas transgenic lines overexpressing TIR1 phenocopy Pi-deprived seedlings [44]. The authors propose that Pi availability modulates LRP number by regulating the auxin sensitivity of the pericycle in a TIR1-dependent manner.

Aux/IAA repressor proteins also dimerize with transcription factors termed auxin response factors (ARFs) that control auxin-responsive transcription [45]. IAA14/SLR has been demonstrated to directly interact with ARF7 and ARF19 [46]. The targeted degradation of IAA14/SLR by auxin and TIR1/AFB1–3 would therefore be expected to cause the de-repression of the transcription factors ARF7 and ARF19 (Figure 3). Consistent with this model, the *arf7 arf19* double mutant phenocopies many of the *slr1* defects, including the disruption of lateral root initiation [31,47]. Nevertheless, unlike *slr1*, the *arf7 arf19* mutant still forms a few lateral roots [10], suggesting that another ARF(s) must also regulate organ initiation. The *ARF* gene family is composed of 23 members [45]; five ARFs have been demonstrated to function as transcriptional activators (ARF4, 5, 6, 7 and 19) and the remaining ARFs seem to behave like transcriptional repressors [48,49]. Several members of the latter class of ARFs also seem to regulate lateral root development [50–52].

Auxin regulates the patterning of new lateral root primordia

Morphologically, lateral root initiation is usually first detected when pairs of pericycle founder cells undergo several rounds of anticlinal divisions [6–8] (Figure 3).

Table 1. Overview of lateral root mutants involved in known molecular or cellular processes during lateral root formation[a]

Molecular/cellular processes	Mutants	Stage	Lateral root phenotype	Refs
Auxin homeostasis	*sur1*	I	Increased LR number	[90]
	sur2	I	Increased LR number	[91]
	ilr2	I	Reduced LR number	[92]
	arf8	I	Increased LR number	[52]
	ilr1 iar3 ill2	I	Reduced LR number	[93]
	ydk1	I	Reduced LR number	[94]
	dfl1	I	Reduced LR number	[95]
Auxin signalling	*tir1 afb1*	I	Reduced LR number	[43]
	axr1	I	Reduced LR number	[96]
	axr6/cul1	I	Reduced LR number	[97]
	iaa1/axr5	I	Reduced LR number on auxin	[33]
	iaa3/shy2-2	I & E	Reduced LR number	[34]
	iaa14/slr	I & E	No LR	[31]
	iaa18/crane	I	Reduced LR number	[35]
	iaa19/msg2	I	Reduced LR number	[36]
	iaa28	I	Strongly reduced or no LR	[37]
	arf 7 arf19; mir160-resistant	I & E	No LR	[32]
	ARF10/16/17	I	Reduced LR number	[50,51]
Auxin transport	*aux1*	I	Reduced LR number	[98]
	lax3	E	Reduced LR number	[67]
	pin1/3/4/7 and combinations	I & P	Reduced LR number	[30]
	pgp4	I	Increased LR number	[99]
Auxin transport protein addressing and regulation	*gnom*	I	Reduced LR number	[56]
	axr4	I	Reduced LR number	[100]
	pinoid	I	Reduced LR number	[101]
	big/tir3/doc1	I	Reduced LR number	[102]
Cell cycle	*35S:KRP2*	I	Reduced LR number	[103]
	alf4	I	No LR	[20]
Cell-to-cell communication	*acr4; crr1/2/3/4* and combinations	I	More LR primordia and disturbed spacing	[54]
Chromatin remodelling	*pickle*	I	Increased LR number on auxin	[104]
Cytokinin pathway	*brx-2*	I	Increased LR number on cytokinin	[88]
F-box proteins	*35S:SINAT5*	I	Reduced LR number	[105]
	cegenduo	I	Increased LR number	[106]
	xbat32-1	I	Reduced LR number	[107]
	arabidillo1/2	I	Reduced LR number	[108]

Abbreviations: E, emergence; I, initiation; P, patterning.
[a]This list is restricted to the small number of mutants for which the molecular/cellular process has been described amongst the high number of mutants with a lateral root defect [9].

Characteristically, these formative cell divisions are asymmetric, giving rise to shorter and longer daughter cells at the centre and flanks of the new LRP, respectively (Figure 3). Asymmetric cell divisions are usually indicative that daughter cells will adopt distinct developmental fates [53]. Consistent with this conclusion, shorter (but not longer) daughter cells express marker genes associated with asymmetric division [54].

Researchers have recently attempted to identify the genes that regulate these formative divisions by transcript profiling *arf7 arf19* and *slr-1* mutants that fail to initiate LRP [31,55]. It was observed that the *slr-1* mutation affected the expression of several cell cycle regulatory genes, among others [55]. Overexpression of the cell cycle regulator *CycD3;1* (which promotes G1 to S phase transition) in the *slr-1* background induced a few rounds of anticlinal divisions [55] but failed to activate the expression of markers such as *ACR4* (*ARABIDOPSIS CRINKLY4*) [54] or lead to the formation of LRP [55]. The activation of cell proliferation is therefore not sufficient to rescue lateral root development in *slr-1*. Instead, pericycle founder cells must seemingly go through asymmetric cell division to express genes such as *ACR4* in their shorter daughter cells [54].

The GNOM protein seems to be required for these initial asymmetric cell divisions [56,57]. Although pericycle cells of weak alleles of *gnom* are still able to proliferate in response to the addition of auxin, they fail to express markers such as ACR4 [54] or form normally patterned LRP [56]. *GNOM* encodes an *ARF-GEF* (*ADP RIBOSYLATION FACTOR-GUANIDINE EXCHANGE FACTOR*) that regulates the trafficking of PIN auxin efflux carrier proteins [57]. Mutants lacking several PIN genes (such as *PIN1, 3, 4* and 7) phenocopy the auxin-induced lateral root phenotype observed in weak *gnom* alleles [58]. This is because GNOM and PIN transporters are required to establish an auxin gradient during primordium formation, with a maximum initially in the central cells then later at the tip of the multilayered LRP (Figure 1). This auxin maximum is likely to pattern the new LRP in a manner similar to a morphogen gradient during animal development [59]. However, regulatory genes such as transcription factors that are expressed at a particular threshold along the auxin response gradient in LRP still wait to be identified.

Auxin, initially accumulating in the central cells of the LRP, then later at the tip of the LRP, will target the degradation of Aux/IAA proteins, enabling ARF proteins to transcribe genes that pattern the new LRP. Recently, two ARF7- and ARF19-regulated genes capable of rescuing the *arf7 arf19* lateral root defect when overexpressed in the

double mutant background were identified [60]. *LBD16/ASL18* (*LATERAL ORGAN BOUNDARIES-DOMAIN16/ASYMMETRIC LEAVES2-LIKE18*) and *LBD29/ASL16* belong to a large family of plant-specific transcription factors. *LBD16/ASL18* and *LBD29/ASL16* promoters represent direct targets for ARF7 and ARF19 [60]. Hence, these LBD proteins seem to function immediately downstream to ARF7 and ARF19 and mediate auxin-regulated lateral root patterning (Figure 3). Identifying which genes are regulated by *LBD16* and *LBD29* is likely to prove crucial to understanding how newly dividing pericycle founder cells give rise to a correctly patterned LRP (Figure 1).

In addition to auxin, cytokinins (CKs) also influence the patterning of new LRP. Targeted expression of the CK biosynthesis gene *IPT* (*ISOPENTENYL TRANSFERASE*) in xylem pole pericycle (but not new LRP) cells was observed to disrupt lateral root initiation and patterning [61]. This result implies that CK interferes with very early patterning events. CK seems to disrupt LRP patterning by interfering with the expression of PIN genes, disrupting the formation of an auxin gradient [61]. A similar mechanism that involves auxin–CK crosstalk has been recently described in the root apical meristem [62]. Consistent with this model, addition of auxin to roots expressing IPT in xylem pole pericycle cells caused them to proliferate but fail to form patterned LRP [61]. Exogenous application of CK leads to abnormal tangential and oblique divisions in stage II LRP, followed by ectopic divisions in the inner layer and rounds of periclinal divisions in the central cells of the outer layer, giving rise to flattened LRP [61]. Interestingly, a similar deviation from the otherwise tightly controlled cellular divisions during LRP organogenesis has been observed in a mutant defective for the AP2 (APETALA2)/EREBP (ETHYLENE RESPONSIVE ELEMENT BINDING PROTEIN) transcription factor *PUCHI* [63].

In addition to auxin and cytokinin, signalling molecules are likely to exist that regulate the asymmetric pericycle cell divisions and thereby influence the patterning of newly initiated LRP. Recently, over a dozen potential regulatory genes that regulate asymmetric cell division and cell fate specification were identified by transcript profiling xylem pole pericycle cells undergoing lateral root initiation [54]. One of these candidates, *ACR4*, was expressed in the small daughter cells after the first asymmetric division in the xylem pole pericycle. Mutants lacking *ACR4* and related gene family member *CRR4* (*CRINKLY4 RELATED4*) form LRP that either fail to express, or exhibit aberrant expression of, the boundary and auxin response markers *LBD5:GUS* and *DR5:GUS*, respectively [54]. ACR4 therefore seems to influence the specification of cell fate during lateral root initiation. *ACR4* encodes a membrane-associated receptor-like kinase [54], raising the intriguing possibility that it regulates LRP patterning by recognizing a novel class of signalling molecule, such as a peptide. ACR4 and its related genes are also required to repress divisions in nearby pericycle cells [54]. Mutants in *ACR4* gene family members cause LRP to initiate close to one another or at the normally excluded opposite poles [54]. Hence, ACR4 forms part of a novel patterning mechanism that exists to repress proliferative divisions in adjacent pericycle cells [54] (Figure 3). Given that ACR4 is expressed exclusively in LRP, the receptor-like protein is likely to regulate a non-cell-autonomous signal(s) that represses division in nearby pericycle cells.

Auxin reprograms cells overlaying LRP to facilitate organ emergence

To emerge, LRP must first pass through several intervening parental root tissues. In *Arabidopsis*, these comprise single layers of endodermal, cortical and epidermal cells (Figure 4). In other plant species, such as rice, LRP must negotiate up to 15 intervening layers of cells [3]. Researchers have speculated for over a century that lateral root development and cell separation processes in adjacent root tissues must be tightly coordinated to minimize tissue damage and reduce the risk of infection (reviewed in Ref. [1]). More recently, genes encoding several *Arabidopsis* cell wall remodelling (CWR) enzymes have been reported to be expressed in root cells next to new LRP, presumably to cause cell separation in overlaying root tissues and promote LRP emergence [64–66].

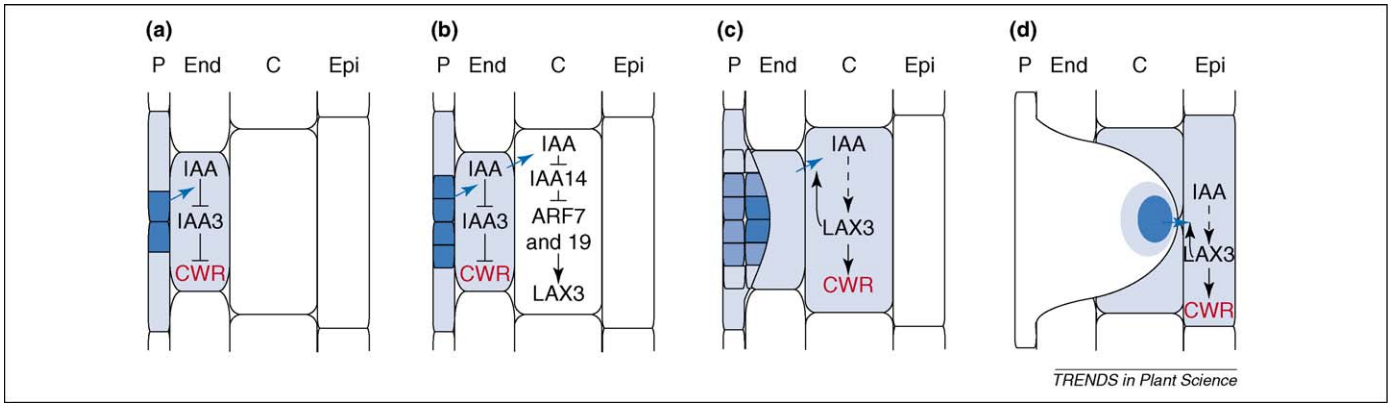

Figure 4. Lateral root emergence. In endodermal cells, the auxin-dependent degradation of the IAA3/SHY2 repressor triggers the expression of selected cell wall remodelling (CWR) enzymes to initiate cell separation **(a)**. Auxin derived from the primordium induces the expression of an auxin influx carrier, LAX3, in adjacent cortical cells after the degradation of the IAA14/SLR repressor **(b)**. The resulting LAX3-dependent accumulation of auxin causes the upregulation of expression of a distinct set of CWR enzymes that initiate cortical cell separation **(c)**. Auxin derived from later stage primordia in closer proximity to the overlaying epidermal cells induces the expression of *LAX3*, and then *CWR* genes **(d)**, causing cell separation and ultimately resulting in lateral root emergence. Hence, emergence of LRP through the outer root tissues is a highly regulated process involving a complex transcellular auxin signalling network. Abbreviations: P, pericycle; End, endodermis; C, cortex; Epi, epidermis. Adapted from Ref. [67].

It was recently reported that auxin originating from developing LRP acts as a local inductive signal that reprograms adjacent cells, causing their cell walls to separate [67]. LAX3 (LIKE AUX1-3) regulates the expression of several CWR enzymes. These include a pectate lyase (PLA2); polygalacturonase (PG); xyloglucan:xyloglucosyl transferase (XTR6); expansin (EXP17); and glycosyl hydrolase (GLH17) [68–72]. The PG and PLA2 enzymes are likely to be particularly important for cell separation during lateral root emergence because they cleave demethylated pectin, a substrate that is enriched in the middle lamella of root cells overlaying LRP. Intriguingly, the endodermal-expressed IAA3/SHY2 protein regulates a set of CWR enzymes that overlaps but is distinct from the set of enzymes regulated by LAX3; this is likely to reflect differences in cell wall composition between lateral and endodermal root tissues.

The restricted pattern of *LAX3* expression in outer root tissues is important for the localized induction of CWR genes such as *PG* and *XTR6*. This regulatory arrangement ensures that the auxin-dependent induction of components of the cell separation machinery is strictly controlled and does not compromise the integrity of the parent root. High levels of auxin are known to cause cell separation in *Arabidopsis* roots [22,73]. However, the authors of these studies observed that cells were sloughed off abnormally, whereas under normal conditions cell separation is highly localized. The model for lateral root emergence (Figure 4) helps to explain how the activities of auxin-regulated CWR enzymes are precisely targeted during lateral root emergence without compromising the integrity of surrounding root tissues.

This study also revealed that the developmental progression and emergence of LRP are strictly coordinated. Mutants that disrupt the lateral root emergence process also impact the developmental progression of LRP, as illustrated by the increased proportion of stage I primordia in *lax3* mutant roots [67]. The endodermis and cortex tissues form concentric circles of cells around the pericycle in which LRP initiate (Figure 1). Targeting cell separation between endodermal and cortical cells overlaying the stage I primordia would help to reduce the tissue tension created by these encircling tissues, enabling meristematic cells to divide periclinally and give rise to a new second layer that will protrude out into the parental root (Figure 4).

The *lax3* mutant, which displays disrupted lateral root emergence, exhibits significantly increased numbers of LRP compared to the wild type [67]. The delay in developmental progression of lateral roots in the *lax3* mutant background is likely to cause leaf-derived auxin (which promotes lateral root emergence [74]) to accumulate to supra-optimal levels in the root pericycle, stimulating the ectopic initiation of new primordia. This hypothesis is consistent with results obtained from stochastic modelling of root branching that revealed feedback regulation between lateral root initiation and emergence [25]. We conclude that lateral root emergence is a highly regulated process employing a common signal to synchronize lateral root development and emergence processes.

Lateral root meristem activation and elongation are dependent on auxin

As the new lateral root protrudes through the epidermal layer, activation of the meristem is thought to occur. Although lateral root meristems have the ability to produce auxin [75], it is not known at which stage the primordium acquires independence from the primary root for auxin supply. The arrest of post-emergence development in the *alf3* mutant can be rescued by exogenous application of auxin or indole precursors [76]. It is therefore possible that the ability to synthesize its own auxin could coincide with or even trigger meristem activation. Nevertheless, acropetal transport of auxin from the shoot and/or primary root sources also seems to be necessary for the continued development and elongation of the lateral root. Perturbation of this acropetal auxin transport stream in the *mdr1* (*multiple drug resistance1*) mutant leads to reduced elongation or arrested lateral roots [77].

Future developments

Our understanding of lateral root development has advanced considerably over the past five years. Recent research has thrown new light on the regulation of lateral root initiation and patterning and provided new insight into the molecular basis of pre- and post-initiation processes, such as priming of founder cells and organ emergence, respectively.

Genetic studies have played, and will continue to play, a vital role in the molecular dissection of lateral root development in *Arabidopsis*. Although a great deal of our current knowledge comes from forward genetics screens, it is becoming increasingly difficult to identify new mutants using this approach because obvious phenotypes are being exhausted from root architecture screens. By contrast, thanks to the availability of high resolution spatiotemporal transcript maps of individual cell types and developmental zones in the root, reverse genetic screens are likely to become much more important [78–80]. For example, the catalogue of genes expressed in the xylem pole pericycle cells [54] and in the outer tissues [66] will greatly aid the identification of new genes regulating lateral root initiation and emergence processes, respectively. Conditional approaches are likely to aid the discovery of novel genes. For example, inducible RNA interference and chemical genetic approaches [81] will aid identification and characterization of genes whose loss-of-function mutations either exhibit embryo lethality or redundancy, respectively. In addition to these new tools and approaches, we need to take advantage of the existing resources for studying primary root development. For example, well-described primary root patterning genes like *SCARECROW* [6], *SHORTROOT* [82], *WOX* [27,83] and *PLETHORA* [84] are expressed during lateral root development, suggesting that these genes might play important roles in patterning LRP. Although over 70 *Arabidopsis* mutants with lateral root defects have been described [9], only a small proportion have been well characterized at the morphological level (Table 1).

Impressive progress has been made in determining the role of auxin during lateral root development. Progress has also recently been made in studying the regulation of

lateral root development by other hormones such as ethylene and abscisic acid [85,86] and nutrient signals like phosphate and nitrate [44,87]. A key challenge will be to integrate these signalling pathways with auxin [88]. The increasing knowledge of pre- and post-initiation events will aid the construction of a regulatory network encompassing all of these signals and their transduction pathways within a multi-cellular context. Building and then validating these regulatory networks will be greatly facilitated by adopting an integrative systems biology approach. Recent efforts to incorporate a modelling approach into *Arabidopsis* root research illustrate the utility of this application [24,26].

Acknowledgements

This work was supported by a Marie Curie Intra-European Fellowship within the 7th European Community Framework Programme (B.P.), the Special Research Fund of Ghent University (pre-doctoral fellowship to B.D.R.), the European Molecular Biology Organization Young Investigator Program (E.B.), Junta de Extremadura (I.C.), Institut de Recherche pour le Développement (L.L.), Agropolis Fondation (n°07024 to L.L. and M.J.B.) and by grants from the Interuniversity Poles of Attraction Program-Belgian Science Policy (P5/13 – M.J.B. and T.B.).

References

1 Lloret, P.G. (2002) Lateral root initiation. In *Plant Roots: The Hidden Hal.* (3rd edn) (Waisel, Y. *et al.*, eds), pp. 127–156, Marcel Dekker
2 Hochholdinger, F. *et al.* (2004) From weeds to crops: genetic analysis of root development in cereals. *Trends Plant Sci.* 9, 42–48
3 Osmont, K.S. *et al.* (2007) Hidden branches: developments in root system architecture. *Annu. Rev. Plant Biol.* 58, 93–113
4 Hochholdinger, F. and Zimmermann, R. (2008) Conserved and diverse mechanisms in root development. *Curr. Opin. Plant Biol.* 11, 70–74
5 Dolan, L. *et al.* (1993) Cellular organisation of the *Arabidopsis thaliana* root. *Development* 119, 71–84
6 Malamy, J.E. and Benfey, P.N. (1997) Organization and cell differentiation in lateral roots of *Arabidopsis thaliana*. *Development* 124, 33–44
7 Casimiro, I. *et al.* (2001) Auxin transport promotes *Arabidopsis* lateral root initiation. *Plant Cell* 13, 843–852
8 Dubrovsky, J.G. *et al.* (2001) Early primordium morphogenesis during lateral root initiation in *Arabidopsis thaliana*. *Planta* 214, 30–36
9 De Smet, I. *et al.* (2006) Lateral root initiation or the birth of a new meristem. *Plant Mol. Biol.* 60, 871–887
10 Fukaki, H. *et al.* (2007) Auxin-mediated lateral root formation in higher plants. *Int. Rev. Cytol.* 256, 111–137
11 Grunewald, W. *et al.* (2007) Developmental biology of roots: one common pathway for all angiosperms? *Int. J. Plant Dev. Biol.* 1, 212–225
12 Nibau, C. *et al.* (2008) Branching out in new directions: the control of root architecture by lateral root formation. *New Phytol.* 179, 595–614
13 Zhang, H. *et al.* (2007) Signalling mechanisms underlying the morphological responses of the root system to nitrogen in *Arabidopsis thaliana*. *J. Exp. Bot.* 58, 2329–2338
14 López-Bucio, J. *et al.* (2003) The role of nutrient availability in regulating root architecture. *Curr. Opin. Plant Biol.* 6, 280–287
15 Parizot, B. *et al.* (2008) Diarch symmetry of the vascular bundle in *Arabidopsis* root encompasses the pericycle and is reflected in distich lateral root initiation. *Plant Physiol.* 146, 140–148
16 Mähönen, A.P. *et al.* (2006) Cytokinin signaling and its inhibitor AHP6 regulate cell fate during vascular development. *Science* 311, 94–98
17 Kurup, S. *et al.* (2005) Marking cell lineages in living tissues. *Plant J.* 42, 444–453
18 Beeckman, T. *et al.* (2001) The peri-cell-cycle in *Arabidopsis*. *J. Exp. Bot.* 52, 403–411
19 Casimiro, I. *et al.* (2003) Dissecting *Arabidopsis* lateral root development. *Trends Plant Sci.* 8, 165–171
20 DiDonato, R.J. *et al.* (2004) *Arabidopsis ALF4* encodes a nuclear-localized protein required for lateral root formation. *Plant J.* 37, 340–353
21 De Smet, I. *et al.* (2007) Auxin-dependent regulation of lateral root positioning in the basal meristem of *Arabidopsis*. *Development* 134, 681–690
22 Boerjan, W. *et al.* (1995) Superroot, a recessive mutation in *Arabidopsis*, confers auxin overproduction. *Plant Cell* 7, 1405–1419
23 Dubrovsky, J.G. *et al.* (2008) Auxin acts as a local morphogenetic trigger to specify lateral root founder cells. *Proc. Natl. Acad. Sci. U. S. A.* 105, 8790–8794
24 Lucas, M. *et al.* (2008) An auxin transport-based model of root branching in *Arabidopsis thaliana*. *PLoS One* 3, e3673
25 Lucas, M. *et al.* (2008) Auxin fluxes in the root apex co-regulate gravitropism and lateral root initiation. *J. Exp. Bot.* 59, 55–66
26 Laskowski, M. *et al.* (2008) Root system architecture from coupling cell shape to auxin transport. *PLoS Biol.* 6, e307
27 Ditengou, F.A. *et al.* (2008) Mechanical induction of lateral root initiation in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. U. S. A.* 105, 18818–18823
28 Ottenschläger, I. *et al.* (2003) Gravity-regulated differential auxin transport from columella to lateral root cap cells. *Proc. Natl. Acad. Sci. U. S. A.* 100, 2987–2991
29 Swarup, R. *et al.* (2005) Root gravitropism requires lateral root cap and epidermal cells for transport and response to a mobile auxin signal. *Nat. Cell Biol.* 7, 1057–1065
30 Benková, E. *et al.* (2003) Local, efflux-dependent auxin gradients as a common module for plant organ formation. *Cell* 115, 591–602
31 Fukaki, H. *et al.* (2002) Lateral root formation is blocked by a gain-of-function mutation in the *SOLITARY-ROOT/IAA14* gene of *Arabidopsis*. *Plant J.* 29, 153–168
32 Okushima, Y. *et al.* (2005) Functional genomic analysis of the *AUXIN RESPONSE FACTOR* gene family members in *Arabidopsis thaliana*: unique and overlapping functions of *ARF7* and *ARF19*. *Plant Cell* 17, 444–463
33 Yang, X. *et al.* (2004) The IAA1 protein is encoded by *AXR5* and is a substrate of SCF(TIR1). *Plant J.* 40, 772–782
34 Tian, Q. and Reed, J.W. (1999) Control of auxin-regulated root development by the *Arabidopsis thaliana SHY2/IAA3* gene. *Development* 126, 711–721
35 Uehara, T. *et al.* (2008) Domain II mutations in CRANE/IAA18 suppress lateral root formation and affect shoot development in *Arabidopsis thaliana*. *Plant Cell Physiol.* 49, 1025–1038
36 Tatematsu, K. *et al.* (2004) *MASSUGU2* encodes Aux/IAA19, an auxin-regulated protein that functions together with the transcriptional activator NPH4/ARF7 to regulate differential growth responses of hypocotyl and formation of lateral roots in *Arabidopsis thaliana*. *Plant Cell* 16, 379–393
37 Rogg, L.E. *et al.* (2001) A gain-of-function mutation in *IAA28* suppresses lateral root development. *Plant Cell* 13, 465–480
38 Weijers, D. *et al.* (2005) Developmental specificity of auxin response by pairs of ARF and Aux/IAA transcriptional regulators. *EMBO J.* 24, 1874–1885
39 Dharmasiri, N. *et al.* (2005) The F-box protein TIR1 is an auxin receptor. *Nature* 435, 441–445
40 Kepinski, S. and Leyser, O. (2005) The *Arabidopsis* F-box protein TIR1 is an auxin receptor. *Nature* 435, 446–451
41 Gray, W.M. *et al.* (2003) *Arabidopsis* SGT1b is required for SCF(TIR1)-mediated auxin response. *Plant Cell* 15, 1310–1319
42 Zenser, N. *et al.* (2001) Auxin modulates the degradation rate of Aux/IAA proteins. *Proc. Natl. Acad. Sci. U. S. A.* 98, 11795–11800
43 Dharmasiri, N. *et al.* (2005) Plant development is regulated by a family of auxin receptor F box proteins. *Dev. Cell* 9, 109–119
44 Pérez-Torres, C.A. *et al.* (2008) Phosphate availability alters lateral root development in *Arabidopsis* by modulating auxin sensitivity via a mechanism involving the TIR1 auxin receptor. *Plant Cell* 20, 3258–3272
45 Guilfoyle, T.J. and Hagen, G. (2007) Auxin response factors. *Curr. Opin. Plant Biol.* 10, 453–460
46 Fukaki, H. *et al.* (2005) Tissue-specific expression of stabilized SOLITARY-ROOT/IAA14 alters lateral root development in *Arabidopsis*. *Plant J.* 44, 382–395

47 Wilmoth, J.C. *et al.* (2005) NPH4/ARF7 and ARF19 promote leaf expansion and auxin-induced lateral root formation. *Plant J.* 43, 118–130
48 Tiwari, S.B. *et al.* (2003) The roles of auxin response factor domains in auxin-responsive transcription. *Plant Cell* 15, 533–543
49 Tiwari, S.B. *et al.* (2004) Aux/IAA proteins contain a potent transcriptional repression domain. *Plant Cell* 16, 533–543
50 Mallory, A.C. *et al.* (2005) MicroRNA-directed regulation of *Arabidopsis* AUXIN RESPONSE FACTOR17 is essential for proper development and modulates expression of early auxin response genes. *Plant Cell* 17, 1360–1375
51 Wang, J.W. *et al.* (2005) Control of root cap formation by microRNA-targeted auxin response factors in *Arabidopsis*. *Plant Cell* 17, 2204–2216
52 Tian, C.E. *et al.* (2004) Disruption and overexpression of *auxin response factor 8* gene of *Arabidopsis* affect hypocotyl elongation and root growth habit, indicating its possible involvement in auxin homeostasis in light condition. *Plant J.* 40, 333–343
53 Scheres, B. and Benfey, P.N. (1999) Asymmetric cell division in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 50, 505–537
54 De Smet, I. *et al.* (2008) Receptor-like kinase ACR4 restricts formative cell divisions in the *Arabidopsis* root. *Science* 322, 594–597
55 Vanneste, S. *et al.* (2005) Cell cycle progression in the pericycle is not sufficient for SOLITARY ROOT/IAA14-mediated lateral root initiation in *Arabidopsis thaliana*. *Plant Cell* 17, 3035–3050
56 Geldner, N. *et al.* (2004) Partial loss-of-function alleles reveal a role for GNOM in auxin transport-related, post-embryonic development of *Arabidopsis*. *Development* 131, 389–400
57 Steinmann, T. *et al.* (1999) Coordinated polar localization of auxin efflux carrier PIN1 by GNOM ARF GEF. *Science* 286, 316–318
58 Friml, J. *et al.* (2003) Efflux-dependent auxin gradients establish the apical–basal axis of *Arabidopsis*. *Nature* 426, 147–153
59 Bhalerao, R.P. and Bennett, M.J. (2003) The case for morphogens in plants. *Nat. Cell Biol.* 5, 939–943
60 Okushima, Y. *et al.* (2007) ARF7 and ARF19 regulate lateral root formation via direct activation of *LBD/ASL* genes in *Arabidopsis*. *Plant Cell* 19, 118–130
61 Laplaze, L. *et al.* (2007) Cytokinins act directly on lateral root founder cells to inhibit root initiation. *Plant Cell* 19, 3889–3900
62 Dello Ioio, R. *et al.* (2008) A genetic framework for the control of cell division and differentiation in the root meristem. *Science* 322, 1380–1384
63 Hirota, A. *et al.* (2007) The auxin-regulated AP2/EREBP gene *PUCHI* is required for morphogenesis in the early lateral root primordium of *Arabidopsis*. *Plant Cell* 19, 2156–2168
64 Neuteboom, L.W. *et al.* (1999) A novel subtilisin-like protease gene from *Arabidopsis thaliana* is expressed at sites of lateral root emergence. *DNA Res.* 6, 13–19
65 Roberts, J.A. *et al.* (2002) Abscission, dehiscence, and other cell separation processes. *Annu. Rev. Plant Biol.* 53, 131–158
66 Laskowski, M. *et al.* (2006) Expression profiling of auxin-treated *Arabidopsis* roots: toward a molecular analysis of lateral root emergence. *Plant Cell Physiol.* 47, 788–792
67 Swarup, K. *et al.* (2008) The auxin influx carrier LAX3 promotes lateral root emergence. *Nat. Cell Biol.* 10, 946–954
68 Marín-Rodríguez, M.C. *et al.* (2002) Pectate lyases, cell wall degradation and fruit softening. *J. Exp. Bot.* 53, 2115–2119
69 Wen, F. *et al.* (2006) Cell separation in roots. *Ann. Plant Rev.* 25, 91–105
70 Vissenberg, K. *et al.* (2005) XTH acts at the microfibril–matrix interface during cell elongation. *J. Exp. Bot.* 56, 673–683
71 Cosgrove, D.J. (2000) Loosening of plant cell walls by expansins. *Nature* 407, 321–326
72 Henrissat, B. (1991) A classification of glycosyl hydrolases based on amino acid sequence similarities. *Biochem. J.* 280, 309–316
73 Laskowski, M.J. *et al.* (1995) Formation of lateral root meristems is a two-stage process. *Development* 121, 3303–3310
74 Bhalerao, R.P. *et al.* (2002) Shoot-derived auxin is essential for early lateral root emergence in *Arabidopsis* seedlings. *Plant J.* 29, 325–332
75 Ljung, K. *et al.* (2005) Sites and regulation of auxin biosynthesis in *Arabidopsis* roots. *Plant Cell* 17, 1090–1104
76 Celenza, J.L. *et al.* (1995) A pathway for lateral root formation in *Arabidopsis thaliana*. *Genes Dev.* 9, 2131–2142
77 Wu, G. *et al.* (2007) Mutations in *Arabidopsis* multidrug resistance-like ABC transporters separate the roles of acropetal and basipetal auxin transport in lateral root development. *Plant Cell* 19, 1826–1837
78 Laplaze, L. *et al.* (2005) GAL4-GFP enhancer trap lines for genetic manipulation of lateral root development in *Arabidopsis thaliana*. *J. Exp. Bot.* 56, 2433–2442
79 Birnbaum, K. *et al.* (2003) A gene expression map of the *Arabidopsis* root. *Science* 302, 1956–1960
80 Brady, S.M. *et al.* (2007) A high-resolution root spatiotemporal map reveals dominant expression patterns. *Science* 318, 801–806
81 Lehar, J. *et al.* (2008) Combination chemical genetics. *Nat. Chem. Biol.* 4, 674–681
82 Scheres, B. *et al.* (1995) Mutations affecting the radial organisation of the *Arabidopsis* root display specific defects throughout the embryonic axis. *Development* 121, 53–62
83 Breuninger, H. *et al.* (2008) Differential expression of *WOX* genes mediates apical–basal axis formation in the *Arabidopsis* embryo. *Dev. Cell* 14, 867–876
84 Aida, M. *et al.* (2004) The *PLETHORA* genes mediate patterning of the *Arabidopsis* root stem cell niche. *Cell* 119, 109–120
85 Ivanchenko, M.G. *et al.* (2008) Ethylene-auxin interactions regulate lateral root initiation and emergence in *Arabidopsis thaliana*. *Plant J.* 55, 335–341
86 Negi, S. *et al.* (2008) Ethylene regulates lateral root formation and auxin transport in *Arabidopsis thaliana*. *Plant J.* 55, 175–187
87 Remans, T. *et al.* (2006) The *Arabidopsis* NRT1. *1 transporter participates in the signaling pathway triggering root colonization of nitrate-rich patches. Proc. Natl. Acad. Sci. U. S. A* 103, 19206–19211
88 Li, J. *et al.* (2009) BREVIS RADIX is involved in cytokinin-mediated inhibition of lateral root initiation in *Arabidopsis*. *Planta* 229, 593–603
89 Beemster, G.T. *et al.* (2003) Cell cycle: the key to plant growth control? *Trends Plant Sci.* 8, 154–158
90 Seo, M. *et al.* (1998) Higher activity of an aldehyde oxidase in the auxin-overproducing *superroot1* mutant of *Arabidopsis thaliana*. *Plant Physiol.* 116, 687–693
91 Barlier, I. *et al.* (2000) The *SUR2* gene of *Arabidopsis thaliana* encodes the cytochrome P450 CYP83B1, a modulator of auxin homeostasis. *Proc. Natl. Acad. Sci. U. S. A* 97, 14819–14824
92 Magidin, M. *et al.* (2003) *ILR2*, a novel gene regulating IAA conjugate sensitivity and metal transport in *Arabidopsis thaliana*. *Plant J.* 35, 523–534
93 Rampey, R.A. *et al.* (2004) A family of auxin-conjugate hydrolases that contributes to free indole-3-acetic acid levels during *Arabidopsis* germination. *Plant Physiol.* 135, 978–988
94 Takase, T. *et al.* (2004) *ydk1-D*, an auxin-responsive *GH3* mutant that is involved in hypocotyl and root elongation. *Plant J.* 37, 471–483
95 Nakazawa, M. *et al.* (2001) *DFL1*, an auxin-responsive *GH3* gene homologue, negatively regulates shoot cell elongation and lateral root formation, and positively regulates the light response of hypocotyl length. *Plant J.* 25, 213–221
96 Lincoln, C. *et al.* (1990) Growth and development of the *axr1* mutants of *Arabidopsis*. *Plant Cell* 2, 1071–1080
97 Hobbie, L. *et al.* (2000) The *axr6* mutants of *Arabidopsis thaliana* define a gene involved in auxin response and early development. *Development* 127, 23–32
98 Bennett, M.J. *et al.* (1996) *Arabidopsis AUX1* gene: a permease-like regulator of root gravitropism. *Science* 273, 948–950
99 Santelia, D. *et al.* (2005) MDR-like ABC transporter AtPGP4 is involved in auxin-mediated lateral root and root hair development. *FEBS Lett* 579, 5399–5406
100 Hobbie, L.J. (2006) Auxin and cell polarity: the emergence of AXR4. *Trends Plant Sci.* 11, 517–518
101 Robert, H.S. and Offringa, R. (2008) Regulation of auxin transport polarity by AGC kinases. *Curr. Opin. Plant Biol.* 11, 495–502
102 Desgagné-Penix, I. *et al.* (2005) The auxin *transport inhibitor response 3* (*tir3*) allele of *BIG* and auxin transport inhibitors affect the gibberellin status of *Arabidopsis*. *Plant J.* 41, 231–242
103 Himanen, K. *et al.* (2002) Auxin-mediated cell cycle activation during early lateral root initiation. *Plant Cell* 14, 2339–2351
104 Fukaki, H. *et al.* (2006) PICKLE is required for SOLITARY-ROOT/IAA14-mediated repression of ARF7 and ARF19 activity during *Arabidopsis* lateral root initiation. *Plant J.* 48, 380–389

105 Xie, Q. *et al.* (2002) SINAT5 promotes ubiquitin-related degradation of NAC1 to attenuate auxin signals. *Nature* 419, 167–170
106 Dong, L. *et al.* (2006) An auxin-inducible F-box protein CEGENDUO negatively regulates auxin-mediated lateral root formation in *Arabidopsis*. *Plant Mol. Biol.* 60, 599–615
107 Nodzon, L.A. *et al.* (2004) The ubiquitin ligase XBAT32 regulates lateral root development in *Arabidopsis*. *Plant J.* 40, 996–1006
108 Coates, J.C. *et al.* (2006) Armadillo-related proteins promote lateral root development in *Arabidopsis*. *Proc. Natl. Acad. Sci. U. S. A.* 103, 1621–1626