Journal of Experimental Botany, Vol. 64, No. 9, pp. 2609–2617, 2013
doi:10.1093/jxb/ert155

Journal of Experimental Botany
www.jxb.oxfordjournals.org

REVIEW PAPER

Lateral root initiation is a probabilistic event whose frequency is set by fluctuating levels of auxin response

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Received 14 February 2013; Revised 2 May 2013; Accepted 3 May 2013

Abstract

The locations in which lateral roots arise are determined by local peaks of auxin response driven by whole-plant physiology. The architecture of a plant root system adapts it to the conditions in which it grows: large shoot systems demand large root systems, and growth in soils that have low or patchy nutrient distributions is often best managed by non-uniform patterns of root branching. It is not surprising then that the regulation of lateral root spacing is responsive to a wide array of stimuli. Molecular genetic studies have outlined a mechanism by which multiple modules of auxin response in specific cell types drive lateral root initiation. These peaks of auxin responsiveness are functionally controlled by the growth of the plant and the changing environmental conditions it experiences. Thus, the process of lateral root initiation, which depends on strong local auxin response, is globally mediated.

Key words: *Arabidopsis*, auxin response, auxin transport, gravitropism, lateral root, rhizotaxis, root architecture.

Introduction

The architecture of plant root systems is highly variable. It depends on many factors including the density of branching and the location(s) in which the branches form. Maintaining flexibility as to where branch roots are positioned allows plants to adapt to a wide range of environmental conditions. This overcomes some of the potential disadvantages of being rooted in place. Flexibility in formation of the root system also allows plants to coordinate the extent of the underground system with the mechanical and nutritional needs of the above-ground portion of the plant. This review focuses on what has been learned about the mechanisms that establish the pattern of lateral root (LR) placement along the main root in the model plant *Arabidopsis thaliana*.

A large body of work has indicated that auxin, a plant hormone that impacts many aspects of plant development, is a primary regulator of lateral root initiation (LRI). Plants that are treated with auxin or that overproduce it form more LRs, while plants that are impaired in auxin signalling form fewer (for a review, see Fukaki *et al.*, 2007). The role of auxin, however, is not simple. Auxin is involved in many of the steps that occur prior to the asymmetric cell divisions that mark the formation of lateral root primordia (LRP) (De Smet *et al.*, 2010). Additionally, both the level of auxin and the plants' ability to respond to it are controlled by events that are distributed throughout the plant body. The probability of forming an LR at any given location along the longitudinal axis of the parent root thus resembles the probability of a crowd gathering for an event at a given place and time. The likelihood varies depending on the ability of the participants to arrive at the location, which in turn depends on fluctuations in traffic that are linked to the size of the city and such discrete events as football games or road construction that alter the flow of traffic in local regions. The tendency to gather is also affected by the presence of ticket-takers, people who interact with those who arrive, and permit the assembly to occur. This review is thus divided into three sections: a first which traces the sequence of molecular events that lead to LR formation in relation to the locations in which they occur, a second that addresses how nutrients regulate the response generally though not exclusively by

Abbreviations: FACS, fluorescence-activated cell sorting; IAA, indole-3-acetic acid (auxin); JA, jasmonic acid; LR, lateral root; LRI, lateral root initiation; LRP, lateral root primordium; MeJA, methyl jasmonate; NPA, naphthylphthalamic acid; XPP, xylem pole pericycle.

altering sensitivity to auxin, and, finally, a consideration of factors that control auxin levels. This last section will emphasize the emerging realization that auxin flow from the shoot and from the root tip varies in time and space. An alternative model that is not based on changes in auxin response is also discussed. Work in the field of LRI has truly blossomed in the past few years and it has not been possible to include all of it; apologies are given to all those whose work has been omitted.

The location of LRI is governed by several clear rules

While the position in which LRs form can and does vary, some aspects of positioning appear to be controlled by fixed rules. These are especially clear in the radial dimension.

Radial positioning is limited to the xylem pole pericycle

LRs arise from the pericycle, a ring of cells just interior to the endodermis. The radial position is restricted to cells that are located adjacent to the large protoxylem elements, the xylem pole pericycle (XPP) (Parizot *et al.*, 2008). These XPP cells form two longitudinal files within the root, each of which has some meristematic properties. When root explants are cultured on shoot-inducing medium, divisions in the XPP give rise to shoot meristems, with longitudinal spacing similar to that of LRP, supporting the view that XPP cells retain a high potential for organogenesis without being entirely committed to LR formation (Atta *et al.*, 2009). Gene expression in the XPP shows a much larger commonality between the XPP and the protoxylem cells than would be expected by chance alone, perhaps reflecting their common origin (Parizot *et al.*, 2012). Together, the data indicate that LRI in unmodified, wild-type roots is restricted to a single cell type.

LRI occurs on the outer, convex side of curves

LRs frequently emerge on curves, and, when they do so, they are always on the outer, convex side (DeSmet *et al.*, 2007; Ditengou *et al.*, 2008; Laskowski *et al.*, 2008; Richter *et al.*, 2009). Unlike many rules in biology that are really just modestly increased tendencies for one outcome over another, the side of a curve on which LRs emerge is essentially invariant, and remains so even in PIN mutants that affect the pattern of auxin transport within the root (Laskowski *et al.*, 2008). This suggests that a robust developmental pathway focuses LRI on the convex side of curves.

Zone of inhibition

Studies on the longitudinal spacing of LRs have identified a minimum distance by which LRP are separated: 300 µm in wild-type roots of the Col-0 ecotype grown on agar plates (Dubrovsky *et al.*, 2006). This minimum distance can be modified by growing wild-type roots in nutrient conditions that enhance expression of *TIR1*. Such roots have LRP that are spaced more closely than usual, even when the roots have few enough LRP that there is plenty of empty space available. Clustering of LRP occurs in both radial and longitudinal dimensions, and overcomes the typical 300 µm zone of inhibition (see Supplementary fig. 1 in Pérez-Torres *et al.*, 2008). These data suggest that the zone of inhibition may be related to the level of auxin response in cells surrounding the LRP. Additional support for this idea comes from the observation that expression of three *PLETHORA* transcription factors, genes that are regulated downstream of auxin and which themselves regulate auxin levels (Galinha *et al.*, 2007), prevent clustering of LRP (Hofhuis *et al.*, 2013).

Searching for the parking lot: the longitudinal origin of LRI

While the crowds that gather at specific locations presumably know why the location was chosen, the rules governing the longitudinal location of LRP formation have remained unclear. It is hypothesized that the location(s) are established by the combined action of two auxin response modules (DeSmet *et al.*, 2010). In the first stage, a group of cells located near the root tip in a region called the basal meristem receives signals that enhance LRI. The result of such signalling may prime patches of cells, giving them an increased probability of giving rise to LRs (DeSmet *et al.*, 2007; Moreno-Risueno *et al.*, 2010). In the second stage, a subset of pericycle cells divide to form LRP. The first visible distinction between the founder cells that undergo this formative division and their neighbours is a local increase in auxin response. This is generally measured by increased signalling from the auxin response promoter DR5::GFP (green fluorescent protein) and occurs in a region that ranges from 2.7 mm to 4.8 mm from the root tip (Dubrovsky *et al.*, 2011). This section begins with a discussion of the potential for auxin response in the root meristem and then traces events associated with LRI shootward from there, as depicted in Fig. 1.

The concentration of endogenous auxin (IAA) in the root was mapped and IAA levels were found to be high in the root meristem, with a maximum in the quiescent centre (QC). Substantially elevated levels extend shootward through the meristem, declining near the start of the elongation zone. Interestingly, the level of auxin in the pericycle is notably lower than in the cells that neighbour it. These data were based on a combination of fluorescence-activated cell sorting (FACS) of 14 lines with tissue-specific promoters that drive expression of GFP, and gas chromatography-selected reaction mode-mass spectroscopy (Petersson *et al.*, 2009). This pattern is a snapshot in time that may fluctuate due to changes in auxin synthesis, degradation, or transport. The entire root including the pericycle appears capable of IAA synthesis and established LRP are sites of auxin synthesis (Ljung *et al.*, 2005), but to date there is

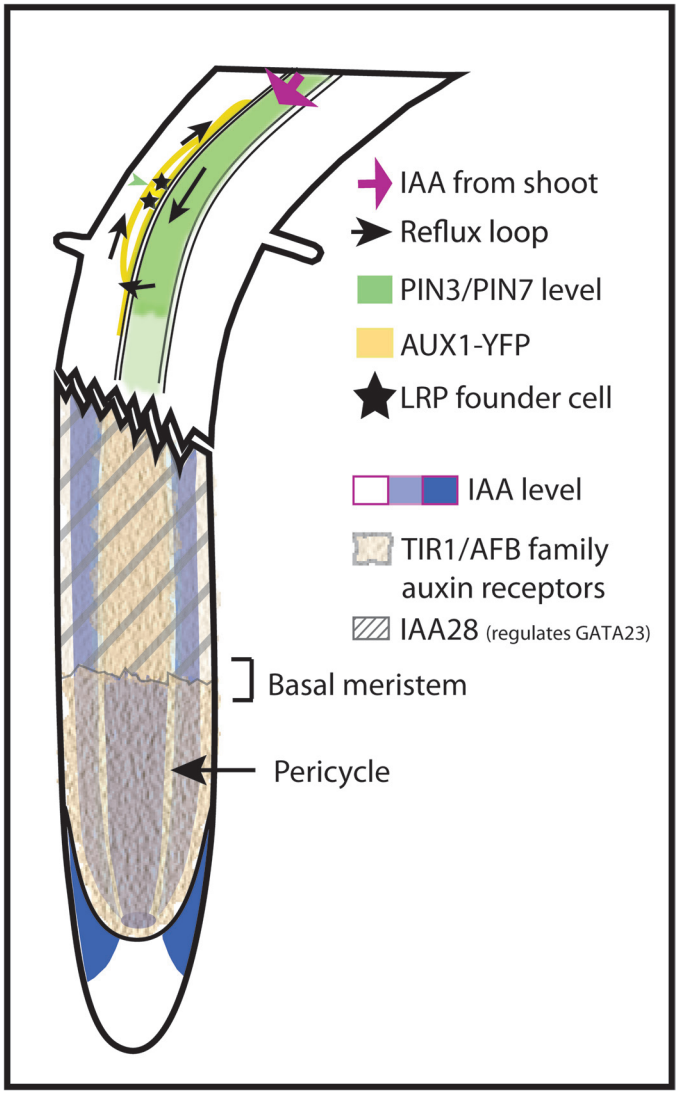

Fig. 1. Diagram showing some of the factors influencing auxin response within the root during the process of lateral root initiation. The bottom section shows the approximate level of IAA (white=low, dark blue=high). This is highest in the meristem and notably low in the pericycle. *TIR1/AFB* auxin receptors (textured regions) are expressed throughout the root, with the highest levels of expression in the meristem and central vasculature, including the pericycle. *IAA28*, whose expression inhibits that of *GATA23*, is present shootward of the meristem. As a member of the AUX/IAA family, it is subject to rapid degradation in response to auxin. The upper section of the drawing shows auxin transport changes associated with founder cell division on gravitropically induced curves. The AUX1 auxin influx protein accumulates in pericycle cells on the outer, convex side and remains present as a subset of those cells undergo radial expansion and division. PIN3 and PIN7 levels are often reduced rootward of the site of founder cell division. The proposed reflux loop that would permit auxin to cycle multiple times through the region is indicated, along with the potential for auxin flow from overlying cell layers.

no evidence to suggest that local auxin synthesis precedes founder cell specification. How then are specific pericycle cells activated?

LRI may follow peaks of auxin response in the basal meristem

De Smet *et al.* (2007) observed peaks of auxin response in the basal meristem that vary with time and correlate with LRP formation. They hypothesized that these pulses of auxin are the initial events that lead to LRI. They also noted that the peaks of auxin response correlate with the waving pattern of growth that their plants displayed. Such waving growth depends on auxin transporters and has been hypothesized to depend on gravity (Oliva and Dunand, 2007); however, roots grown in microgravity still retain a wavy growth pattern (Paul *et al.*, 2012). It will be interesting to see if light, gravity, circadian rhythms, or other factors affect the observed pulses of auxin response and LRI.

The idea that LRI begins with pulses of auxin in the basal meristem is supported by the fact that the spatial pattern of LRP formation depends on TIR1/AFB family auxin receptors. These F-box proteins work in conjunction with the $SCF^{TIR/AFB}$ E3 ubiquitin ligase to target and degrade specific proteins in response to auxin. Degradation of AUX/IAA proteins liberates auxin response factors (ARFs) that then serve as transcriptional regulators (for a review, see Chapman and Estelle, 2009). Transcriptional fusions have shown that the *TIR1*, *AFB1*, *AFB2*, and *AFB3* auxin receptors are expressed at high levels in the root tip near the QC. Expression of *TIR1*, *AFB2*, and *AFB3* trails off near the basal meristem, while expression of *AFB1* remains substantial throughout the root, with the highest levels in the central vasculature (Parry *et al.*, 2009; Supplementary fig. 6 in Pérez-Torres *et al.*, 2008). Treating roots with 1-naphthaleneacetic acid (NAA), a synthetic auxin that can enter cells without the aid of auxin input carriers, confirms that there is a gradient of responsiveness. After addition of NAA, LRP form along the entire length of the root, but are most concentrated in the basal meristem (De Smet *et al.*, 2007). In *tir1afb2 afb3* triple mutants some LRP still form, but the tendency to form in the region closest to the root tip is diminished and the variance in the longitudinal position at which they appear is dramatically wider than in the wild type (Dubrovsky *et al.*, 2011). Taken together, these data suggest that events leading to the initiation of LRP occur near the end of the meristem in a region where *TIR1/AFB* receptor expression is high.

In separate studies, expression of the LRI-inducing transcription factor *GATA23* has been shown to occur in patches in the XPP just shootward of the meristem, prior to founder cell division (De Rybel *et al.*, 2010). These patches arise some hours after the peaks of DR5 expression that are observed in synchronously germinated plants, and are blocked by treatment with an inhibitor of TIR1-based signalling, PEO-IAA. This suggests that expression of *GATA23* depends on pulses of auxin response in the basal meristem. Pulses of auxin may lead to *GATA23* expression by degrading IAA28. *IAA28* is expressed in the basal meristem and its stabilized form inhibits LRI (Rogg *et al.*, 2001; De Rybel *et al.*, 2010). Overexpression of *GATA23* behind the XPP-specific GAL4 driver J0121 increases the density of LRP and can lead to formation of clusters of LRs, indicating that expression of *GATA23* plays a role in LRI.

Oscillatory production of pre-branch sites

A fascinating alternative model is that LRI is controlled by regular, periodic production of pre-branch sites, which are described as groups of cells that have the capacity to form LRP (Moreno-Risueno *et al.*, 2010). These sites are defined by oscillating waves of gene expression. Evidence for the model is drawn from microarray experiments showing that when the primary root is carefully dissected, separate pieces cut from the same relative location exhibit distinct gene expression profiles. The expression level of the auxin response reporter *DR5* was determined in each of the root segments, and clusters of genes whose high or low expression correlates with *DR5* were observed. Surprisingly, the group of genes whose expression correlates with *DR5* is not highly enriched in genes known to be involved in LR formation (Moreno-Risueno *et al.*, 2010). It will be interesting to see how these results fit with the proposal being made here.

Events occurring at the site of founder cell division

The first indication that founder cell division is about to occur is the accumulation of a strong auxin response maximum, reported as a peak in *DR5* expression. This increase in auxin response occurs coordinately with accumulation of the auxin import facilitator AUX1–YFP (yellow fluorescent protein). Because expression of the auxin importer AUX1 is enhanced by auxin, an initial increase in auxin import sets up a positive feedback loop that drives formation of a localized auxin maximum (Laskowski *et al.*, 2008). When LRI is induced by gravitropic curvature, enhanced expression of AUX1–YFP occurs in patches of the pericycle on the outer side of curves. These patches of AUX1–YFP-expressing cells are longer than the patch of founder cells that divide soon thereafter, but become concentrated around the founder cells with time (Laskowski *et al.*, 2008).

Although formation of an auxin maximum may usually follow the priming or specification events described above, any treatment that results in high levels of auxin in the pericycle in this region can result in LRI. This was shown by older experiments in which exogenous treatment activated LRI throughout the root (Laskowski *et al.*, 1995), and more recently by elegant experiments in which a clonal activation system was used to drive expression of the auxin biosynthetic enzyme *iaaM* in small patches of pericycle cells (Dubrovsky *et al.*, 2008). The number of cells with enhanced auxin biosynthetic capacity corresponded to the extent by which LRI increased. These treatments also disrupted the regular pattern of LRI, permitting LRP to form more closely to one another than normally occurs. This indicates that a large number of XPP cells are competent to respond to auxin, though only some of them are regularly triggered to form auxin maxima.

Formation of a peak of *DR5* expression is under control of *GNOM*/*FEWER ROOTS* (Okumura *et al.* 2013). The partial loss-of-function mutant *fwr* has elevated levels of free IAA and shows expression of *DR5* throughout wide stretches of the root vasculature, but these roots fail to produce strong, local peaks of *DR5* and formation of LRP is severely impaired. Although *GNOM* has been shown to regulate PIN1 in the embryo, no change in PIN1–GFP expression was seen in roots of *fwr*. Primary root growth is unaffected in these mutants, suggesting that the effect is specific to LRI (Okumura *et al.*, 2013). Further examination of these lines may reveal important mechanisms underlying the positioning of LR formation.

Once an auxin maximum has been established, several factors interact, leading to nuclear migration and asymmetric division of the founder cells. Auxin-mediated destruction of the transcriptional repressor SOLITARY-ROOT/IAA 14 initiates an ARF-based transcriptional cascade (for a review, see Fukaki *et al.*, 2007). Degradation of SOLITARY ROOT/IAA14 activates ARF7 and ARF19, which directly target LBD transcription factors (Okushima *et al.*, 2007). Plants carrying loss-of-function mutations in both *ARF7* and *ARF19* make few LRs; however, expressing *LBD16* in the XPP of *arf7,arf19* partially rescues LR formation, strongly supporting the idea that *LBD16* regulates LRI (Goh *et al.*, 2012*a*). Additional *LBD* genes likewise participate in the early stages of LR formation (Lee *et al.*, 2009; Berckmans *et al.*, 2011; Feng *et al.*, 2012*a*, *b*).

Asymmetric cell division in the founder cells can also be regulated by small peptides in the CLE-like family. Overexpression of the CLE-like peptides *CLEL6* or *CLEL7* inhibits the regular pattern of asymmetric cell division leading to LRP formation (Meng *et al.*, 2012). Though *CLEL6* and *CLEL7* may not be expressed in XPP early enough to be involved in the first asymmetric cell division, other related family members are expressed in stage I LRP (Fernandez *et al.*, 2013), and family members may well act in a redundant fashion. Peptide signalling may act in concert with transcriptional regulation to organize this stage of LRI.

Coincident with founder cell division, three *PLT* genes, *PLT3*, *PLT5*, and *PLT7*, become expressed. Inducible overexpression of these in *arf7, arf19* also rescues LR formation (Hofhuis *et al.*, 2013). Thus members of the *LBD*, *CLEL*/*GLV*, and *PLT* gene families are all early effectors of LRI.

Environmental conditions can regulate LRI by modulating auxin response

The model for rhizotactic patterning described here assumes that the auxin response is the central component that establishes new LRs. The incredible ability that plants have to respond to their environment allows us to test our hypotheses by determining how environmental influences interact with the developmental pathways. In the case of LRI, there is ample support for the centrality of the auxin response as the key regulatory factor.

Phosphate

Root architecture is strongly influenced by the level of available phosphate. In low phosphate conditions, elongation of the primary root is inhibited while LRI and LR elongation

are promoted, resulting in a root system that can more efficiently explore the upper reaches of the soil. These upper regions tend to contain higher levels of available phosphate (for reviews, see Péret *et al.*, 2011; Niu *et al.*, 2012). The promotion of LRI by low phosphate is notable in that it occurs in the absence of altered auxin levels (Pérez-Torres *et al.*, 2008). Instead, expression of the auxin receptor *TIR1* is enhanced in the central vasculature of the root including the pericycle. Loss-of-function mutants of *tir1* do not exhibit increased LRI in response to low phosphate, indicating that *TIR1* is required for the phosphate response (Pérez-Torres *et al.*, 2008). Increased *TIR1* expression in response to low phosphate is a prime example of an environmentally regulated increase in auxin sensitivity.

Nitrate

The plant response to nitrate is complex, involving both systemic and local reactions, but adding nitrate to plants growing in a low nitrate environment increases the total density of LRs. In parallel to the response to low phosphate, the increase in LRI depends on enhanced expression of a *TIR1/AFB* auxin receptor, *AFB3*. Increased expression in response to nitrate is specifically seen for *AFB3* and not other individual members of the *TIR1/AFB* family (Vidal *et al.*, 2010). Thus enhanced levels of *AFB3* increase the probability of inducing an LR by increasing auxin sensitivity.

General nutritional status

In general, the nutrient status of the plant is related to the density of LRI, as shown by multiple experiments in which LR formation correlates with enhanced sucrose supply (for a review, see Roycewicz and Malamy, 2012). In a recent survey of gene expression, about two-thirds of the known auxin-regulated genes were found to be regulated by glucose, raising the possibility that the effect of glucose on LRI acts in part through changes in auxin response (Mishra *et al.*, 2009); however, further work is needed to determine if the response to glucose acts through any of these changes or if the changes in expression are also the result of a general nutritional enhancement.

The recent observation that IAA18 can move across a graft junction (Notaguchi *et al.*, 2012) raises the intriguing possibility that the shoot and root growth could be interconnected via movement of signalling molecules in the phloem. Expression of *IAA18* inhibits LRI, probably by inhibiting the action of ARF7 and ARF19 (Uehara *et al.*, 2008). Grafting pieces of shoot tissue that express the stabilized *crane-2/iaa18* to wild-type rootstocks results in fewer LRs in a 3 cm long region near the root tip than are formed when similar grafts are made with wild-type scions (Notaguchi *et al.*, 2012). Because the rate of phloem transport depends on source–sink relationships between the root and shoot, a phloem-mobile inhibitor of LRI has the potential to integrate LRI with shoot growth as cargo from the phloem is unloaded in growing regions of the root.

Auxin concentrations in the zone of LRI are mediated by whole-plant physiology

Nutritional and environmental conditions modulate the level of auxin within the root, as well as the ability to respond to it. For example, wild-type roots respond to high osmotic conditions with increased levels of free IAA and increased numbers of emerged LRs (Kinoshita *et al.*, 2012). This increase in free auxin depends on up-regulation of the auxin de-conjugating enzyme *IAA-Ala Resistant3* (*IAR3*), which occurs as a result of a reduction in accumulation of the microRNA *miR167a/b* that targets it (Kinoshita *et al.*, 2012).

Movement of auxin from shoot to root impacts LRI

Auxin transport from the shoot to the root promotes both LRI and elongation. Early experiments showed that radioactive auxin applied to the shoot accumulates at sites of LR formation (Rowntree and Morris, 1979), and extensive experiments have confirmed that the flow of auxin from the shoot has a large effect on LR emergence (Bhalerao *et al.*, 2002). That shoot-derived auxin also impacts LRI is suggested by classic cotyledon-removal experiments which decrease the number and density of LRP (Wightman and Thimann, 1980; Hinchee and Rost, 1986). Furthermore, treating plants with the auxin transport inhibitor naphthylphthalamic acid (NPA) at the root–shoot junction or at points along the roots results in a dramatic reduction in emerged LRs rootward of the application site (Reed *et al.*, 1998). These observations gave rise to the general view that auxin moves from the shoot through the central vasculature of the primary root toward the root tip as a single, continuous stream.

The level of auxin reaching the root varies strongly with the age of the plant. A pulse of auxin has been observed reaching the root between 5 d and 7 d after germination (Bhalerao *et al.*, 2002). Interestingly, there is a rough correspondence between auxin levels, which increase with age up to at least 8 d after germination (Bhalerao *et al.*, 2002), and the density of LRP, which also increases with age of the root (Lucas *et al.*, 2008*a*).

Auxin levels also vary with the curvature of the root. Increases in the level of *DR5::GFP* expression on the outer side of the central vasculature were observed in response to curving roots (Laskowski *et al.*, 2008), and this correlates with the increased presence of emerged LRs on the outside of curves. Computer simulations in which auxin flowing from the shoot moves through a network of auxin transporters approximating that observed in the root have shown that curves may affect auxin flow by increasing the opportunity for lateral transport. Such an effect could potentially lead to the formation of a reflux loop in which the same molecules of auxin flow through the curved region several times before moving on to more rootward sections. This could explain the observed increase in auxin response in curved regions and would be expected to have a promoting effect on LR emergence as well as LRI (Laskowski *et al.*, 2008).

Indeed, recent work supports the idea that auxin flow from cells overlying the pericycle may contribute to founder cell

division (Marhavý *et al.*, 2013). PIN3 is specifically expressed in endodermal cells that overlie regions of the pericycle with enhanced levels of *DR5::GFP* expression. To test whether lateral transport from the endodermis to the pericycle could contribute to LRI, roots were manually curved and the time to subsequent events was measured. Loss-of-function *pin3* mutants are delayed in the formation of strong auxin maxima that precede nuclear migration. The first asymmetric division of the founder cells is also delayed. Expressing *PIN3* behind the *SCR* promoter, which is expressed throughout the endodermis, largely rescues this *pin3*-mediated delay in LR development. Thus, auxin flow from the cell layer external to the pericycle probably contributes to the peak of auxin response that leads to founder cell division (Marhavý *et al.*, 2013).

The fact that mechanical manipulation increases LRI raised the possibility that the increase in auxin response on the outside of curves could be potentiated by mechanosensing (Ditengou *et al.*, 2008; Laskowski *et al.*, 2008; Richter *et al.*, 2009). The formation of emerged LRs on the convex side of a curve has now been shown to require a functional version of the jasmonate receptor *COI1* (Raya-Gonzalez *et al.*, 2012). Although the plant hormone jasmonate is best known for its role in plant defence, application of the methyl ester of jasmonate (MeJA) alters the localization of auxin transporters, induces several genes involved in auxin biosynthesis, and results in enhanced LRI (Sun *et al.*, 2009, 2011). The jasmonate receptor *COI1* is also required for several touch-mediated responses in the shoot, including the inhibition of elongation of *Arabidopsis* inflorescences. In that case, the same stimulus that affects the response also leads to increased levels of JA (Chebab *et al.*, 2012). If a similar response pathway existed in roots, then the increased elongation of cells on the convex side of a curve could potentiate an increase in mechanosensing, leading to JA production and subsequent LRI.

Another example in which heterogeneity of rootward auxin transport may contribute to LRI is found in the auxin efflux carriers PIN3 and PIN7 that exhibit discontinuities in expression within the central vasculature of the primary root. The level of fluorescence from PIN3–GFP and PIN7–GFP labelled fusion proteins is frequently lower on the rootward side of a curve than the shootward side (Laskowski *et al.*, 2008; Lewis *et al.*, 2011). Such decreases in PIN expression could lead to accumulation of auxin on the shootward side of the discontinuity. Treatment with the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) increases auxin transport from the shoot toward the root in a PIN3- and PIN7-dependent manner, and raises the expression level of these fluorescently tagged proteins such that they exhibit uniformly high expression. ACC also inhibits LRI, suggesting that the discontinuity of PIN expression in the vasculature might influence LRI (Lewis *et al.*, 2011).

This more nuanced view of the effect of the rootward transport stream does not necessarily conflict with the classical view that auxin transport into the root increases LRI. For example, the auxin-responsive gene *ABI4* inhibits the rootward auxin transport stream, possibly through PIN1 whose expression level correlates with *ABI4* expression, and in which no discontinuities were reported. *abi4* loss-of-function mutants have more densely spaced LRP, as would be expected if the auxin level in the primary root were uniformly increased (Shkolnik-Inbar and Bar-Zvi, 2010).

A growing body of evidence further suggests that the distribution of LRs may be affected by the development of pre-existing LRP. Several mutants that are impaired in early stages of LR outgrowth display increased LRP densities, and this is often accompanied by the appearance of closely spaced clusters of LRP (De Smet *et al.*, 2008; Swarup *et al.*, 2008; Goh *et al.*, 2012*a*, *b*; Hofhuis *et al.*, 2013). This basic correlation is also seen in untreated wild-type plants. The average distance separating two emerged LRs is larger than the distance around LRP that are delayed in emergence (Lucas *et al.*, 2008*b*). The cause of this correlation between LRP outgrowth and LRP spacing is not clear. The failure of LRP to develop may somehow increase the tendency to form subsequent LRP. This possibility is consistent with the observation that gain-of-function *shy2-101* plants, which are impaired in LR emergence, show large increases in the total amount of free IAA within the root (Goh *et al.*, 2012*b*). Alternatively, the tendency of LRs to emerge could be enhanced when they arise far from existing LRP. Either way, the observations suggest that LRP may themselves affect subsequent LRs.

Shootward auxin transport

Auxin is transported from the root tip through the epidermal and cortical cell layers toward the end of the elongation zone (Blilou *et al.*, 2005), and it has been hypothesized that this shootward transport stream plays a role in LRI. Mutant *stm* seedlings that lack a shoot meristem produce a density of LRs similar to that of the wild type, which suggests that LRI is induced by a root-based auxin supply (Casimiro *et al.*, 2001). Further experimentation is required to determine the extent to which auxin coming from the shoot is actually reduced, as earlier experiments in pea showed that the cotyledons were a primary factor in maintaining LR density in the early stages of seedling growth (Wightman and Thimann, 1980). Several plants with impaired LRI carry mutations in genes that are expressed in the root cap (Moreno-Risueno *et al.*, 2010), a fact that may support the importance of such a transport stream.

LRI is enhanced by gravitropic stimuli

Although questions about the general mechanism of LRI remain, there is a broad consensus that gravitropic stimuli induce LRI (DeSmet *et al.*, 2007; Ditengou *et al.*, 2008; Laskowski *et al.*, 2008; Lucas *et al.*, 2008*b*; Moreno-Risueno *et al.*, 2010). Lucas *et al.* (2008*b*) showed that the magnitude of LRI varies depending on the interval between gravitropic inductions, with intervals of 6 h leading to LRI in nearly 100% of cases. The fluctuation in responsiveness may reflect changes in auxin response. The root cap might act as a capacitor, accumulating some factor for a period of time before releasing it all at once in response to gravitropic stimulation. In such a case, the amount of response would depend on

the level of the LRI-promoting factor accumulated prior to gravistimulation (Lucas *et al.*, 2008*b*).

More recently, the pattern of auxin flow in the root tip has been shown to undergo a sharp transition during the gravitational response. Auxin response was followed by a fluorescent reporter fused to the DII domain from Aux/IAA proteins. This domain, and hence the entire reporter, is rapidly degraded in response to auxin (Brunoud *et al.*, 2012). Surprisingly, the differential in auxin response on the two sides of a gravitropically stimulated root suddenly disappeared when the root reached ~45 ° curvature (Band *et al.*, 2012). Immediately after the roots are placed in a horizontal orientation, the statoliths, which are large, relatively heavy plastids in the columella cells, came to rest on the bottom side of the root. As the root curves, the surface that these objects rested on acquired an increasingly steep slope and, when the curvature of the root reached ~45 °, they fell to the new bottom, which was then closest to the root tip. It was proposed that this change in the location of the statoliths may initiate auxin redistribution (Band *et al.*, 2012), and one possibility is that balancing auxin levels on the two sides of the root may occur via a pulse of auxin moving toward the outer, convex side of curves in or near the basal meristem. Regardless of the mechanism, the redistribution of auxin that occurs around this degree of curvature is remarkable in the suddenness of its onset.

Both auxin redistribution in the root tip and the formation of LRs that occurs in response to gravity exhibit an unusually sharp threshold at the same degree of root curvature. Roots curved >45 ° essentially always form new LRs at the site of the bend, whereas roots curved just <45 ° have essentially no effect on LRI (compare fig. 1E in Laskowski *et al.*, 2008 with fig. 3E in Band *et al.*, 2012). This raises the possibility that these events may be causally linked. Gravitropically induced pulses of auxin movement could be synonymous with at least some of the pulses of auxin response seen in the basal meristem (DeSmet *et al.*, 2007; Moreno-Risueno *et al.*, 2010). The details of such an auxin flow are far from worked out; indeed, it is still possible that the redistribution of auxin occurs as a result of auxin destruction on the lower side of the root rather than movement toward the upper side, but the potential for synthesis is intriguing and deserves future attention.

Conclusions and future perspectives

A large body of evidence supports a model whereby the longitudinal positioning of LRI is a probabilistic event whose frequency is specified by the combination of auxin levels and auxin responsiveness in pericycle cells within the region of LRI. Events occurring in a variety of locations in the plant may lead to pulses of auxin response in the basal meristem that trigger subsequent progression toward founder cell division. Whether as a result of these or other mechanisms, the creation of an auxin maximum near the shootward end of the elongation zone then suffices to permit the asymmetric divisions of the pericycle that lead to LRI. Whether the environmental effects that impact LRI exert their influence through changes in auxin response in the basal meristem or through local effects in the region of founder cell division remains unknown for many cases, and it will be interesting to see what the answers are. In general, however, environmental conditions modulate the tendency to form LRI primarily by altering the extent to which plants respond to existing auxin signals.

It will be exciting to discover how the rules for LRI relate to those of organ formation in other contexts. To what extent is the formation of adventitious roots, roots that initiate from locations other than the main root, controlled by the same or similar gene families? Because adventitious roots do not form close to the basal meristem, some differences between LRI and adventitious root formation might be expected. Determining where the similarities lie can shed light on the extent to which the process of organ formation is conserved and may provide insights that could impact methods used for plant propagation and agriculture. Likewise, it is interesting to compare LRI with the formation of new organs at the shoot apical meristem. In contrast to LRI, which is highly responsive to environmental inputs, the phyllotactic patterning of shoots is comparatively stable. The processes that pattern the root and shoot do share a common basis in that formation of both leaf and root primordia is promoted by auxin. Nonetheless, given the differences in the contexts in which they occur, it was surprising that a recent study identified a common set of transcription factors involved in both rhizotaxis and phyllotaxis. Determining the extent to which the mechanisms that regulate rhizotactic and phyllotactic patterning are conserved will be a fascinating area of future discovery.

Acknowledgements

Work in the author's laboratory was funded by NSF IOS-0950866. Thanks also are given to Ben Scheres, Stephen Grigg, and Hugo Hofhuis for helpful comments.

References

Atta R, Laurens L, Boucheron-Dubuisson E, Guivarc'h A, Carnero E, Giraudat-Pautot V, Rech P, Chriqui D. 2009. Pluripotency of Arabidopsis xylem pericycle underlies shoot regeneration from root and hypocotyl explants grown *in vitro*. *The Plant Journal* **57,** 626–644.

Band LR, Wells DM, Larrieu A, *et al.* 2012. Root gravitropism is regulated by a transient lateral auxin gradient controlled by a tipping-point mechanism. *Proceedings of the National Academy of Sciences, USA* **109,** 4668–4673.

Berckmans B, Vassileva V, Schmid SP, *et al.* 2011. Auxin-dependent cell cycle reactivation through transcriptional regulation of Arabidopsis *E2Fa* by lateral organ boundary proteins. *The Plant Cell* **23,** 3671–3683.

Bhalerao RP, Eklöf J, Ljung K, Marchant A, Bennett M, Sandberg G. 2002. Shoot-derived auxin is essential for early lateral root emergence in Arabidopsis seedlings. *The Plant Journal* **29,** 325–332.

Blilou I, Xu J, Wildwater M, Willemsen V, Paponov I, Friml J, Heidstra R, Aida M, Palme K, Scheres B. 2005. The PIN auxin

efflux facilitator network controls growth and patterning in Arabidopsis roots. *Nature* **433,** 39–44.

Brunoud G, Wells DM, Oliva M, *et al.* 2012. A novel sensor to map auxin response and distribution at high spatio-temporal resolution. *Nature* **482,** 103–106.

Casimiro I, Marchant A, Bhalerao RP, *et al.* 2001. Auxin transport promotes Arabidopsis lateral root initiation. *The Plant Cell* **13,** 843–852.

Chapman EJ, Estelle M. 2009. Mechanism of auxin-regulated gene expression in plants. *Annual Review of Genetics* **43,** 265–285.

Chehab EW, Yao C, Henderson Z, Kim S, Braam J. 2012. Arabidopsis touch-induced morphogenesis is jasmonate mediated and protects against pests. *Current Biology* **22,** 701–706.

De Rybel B, Vassileva V, Parizot B, *et al.* 2010. A novel aux/IAA28 signaling cascade activates GATA23-dependent specification of lateral root founder cell identity. *Current Biology* **20,** 1697–1706.

De Smet I, Lau S, Voss U, *et al.* 2010. Bimodular auxin response controls organogenesis in Arabidopsis. *Proceedings of the National Academy of Sciences, USA* **107,** 2705–2710.

De Smet I, Tetsumura T, De Rybel B, *et al.* 2007. Auxin-dependent regulation of lateral root positioning in the basal meristem of Arabidopsis. *Development* **134,** 681–690.

De Smet I, Vassileva V, De Rybel B, *et al.* 2008. Receptor-like kinase ACR4 restricts formative cell divisions in the Arabidopsis root. *Science* **322,** 594–597.

Ditengou FA, Teale WD, Kochersperger P, *et al.* 2008. Mechanical induction of lateral root initiation in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences, USA* **105,** 18818–18823.

Dubrovsky JG, Sauer M, Napsucialy-Mendivil S, Ivanchenko MG, Friml J, Shishkova S, Celenza J, Benková E. 2008. Auxin acts as a local morphogenetic trigger to specify lateral root founder cells. *Proceedings of the National Academy of Sciences, USA* **105,** 8790–8794.

Dubrovsky JG, Napsucialy-Mendivil S, Duclercq J, Cheng Y, Shishkova S, Ivanchenko MG, Friml J, Murphy AS, Benková E. 2011. Auxin minimum defines a developmental window for lateral root initiation. *New Phytologist* **191,** 970–983.

Dubrovsky JG, Gambetta GA, Hernández-Barrera A, Shishkova S, González I. 2006. Lateral root initiation in Arabidopsis: developmental window, spatial patterning, density and predictability. *Annals of Botany* **97,** 903–915.

Feng Z, Sun X, Wang G, Liu H, Zhu J. 2012*a*. LBD29 regulates the cell cycle progression in response to auxin during lateral root formation in *Arabidopsis thaliana*. *Annals of Botany* **110,** 1–10.

Feng Z, Zhu J, Du X, Cui X. 2012*b*. Effects of three auxin-inducible LBD members on lateral root formation in *Arabidopsis thaliana*. *Planta* **236,** 1227–1237.

Fernandez A, Drozdzecki A, Hoogewijs K, Nguyen A, Beeckman T, Madder A, Hilson P. 2013. Transcriptional and functional classification of the GOLVEN/ROOT GROWTH FACTOR/CLE-like signaling peptides reveals their role in lateral root and hair formation. *Plant Physiology* **161,** 954–970.

Fukaki H, Okushima Y, Tasaka M. 2007. Auxin-mediated lateral root formation in higher plants. *International Review of Cytology* **256,** 111–137.

Galinha C, Hofhuis H, Luijten M, Willemsen V, Blilou I, Heidstra R, Scheres B. 2007. PLETHORA proteins as dose-dependent master regulators of Arabidopsis root development. *Nature* **449,** 1053–1057.

Goh T, Joi S, Mimura T, Fukaki H. 2012*a*. The establishment of asymmetry in Arabidopsis lateral root founder cells is regulated by LBD16/ASL18 and related LBD/ASL proteins. *Development* **139,** 883–893.

Goh T, Kasahara H, Mimura T, Kamiya Y, Fukaki H. 2012*b*. Multiple AUX/IAA-ARF modules regulate lateral root formation: the role of Arabidopsis SHY2/IAA3-mediated auxin signaling. *Philosophical Transactions of the Royal Society B: Biological Sciences* **367,** 1461–1468.

Hofhuis H, Laskowski M, Du Y, Prasad K, Grigg S, Pinon V, Scheres B. 2013. Phyllotaxis and rhizotaxis in Arabidopsis are modified by three *PLETHORA* transcription factors. *Current Biology* (in press).

Hinchee MAW, Rost TL. 1986. The control of lateral root development in cultured pea seedlings. I. The role of seedling organs and plant growth regulators. *Botanical Gazette* **147,** 137–147.

Kinoshita N, Wang H, Kasahara H, Liu J, Macpherson C, Machida Y, Kamiya Y, Hannah MA, Chua NH. 2012. *IAA-Ala Resistant3*, an evolutionarily conserved target of miR167, mediates Arabidopsis root architecture changes during high osmotic stress. *The Plant Cell* **24,** 3590–3602.

Laskowski M, Grieneisen VA, Hofhuis H, Hove CA, Hogeweg P, Marée AF, Scheres B. 2008. Root system architecture from coupling cell shape to auxin transport. *PLoS Biology* **6,** e307.

Laskowski MJ, Williams ME, Nusbaum HC, Sussex IM. 1995. Formation of lateral root meristems is a two-stage process. *Development* **121,** 3303–3310.

Lee HW, Kim NY, Lee DJ, Kim J. 2009. *LBD18/ASL20* regulates lateral root formation in combination with *LBD16/ASL18* downstream of *ARF7* and *ARF19* in Arabidopsis. *Plant Physiology* **151,** 1377–1389.

Lewis DR, Negi S, Sukumar P, Muday GK. 2011. Ethylene inhibits lateral root development, increases IAA transport and expression of PIN3 and PIN7 auxin efflux carriers. *Development* **138,** 3485–3495.

Ljung K, Hull AK, Celenza J, Yamada M, Estelle M, Normanly J, Sandberg G. 2005. Sites and regulation of auxin biosynthesis in Arabidopsis roots. *The Plant Cell* **17,** 1090–1104.

Lucas M, Godin C, Jay-Allemand C, Laplaze L. 2008*b*. Auxin fluxes in the root apex co-regulated gravitropism and lateral root initiation. *Journal of Experimental Botany* **59,** 55–66.

Lucas M, Guédon Y, Jay-Allemand C, Laplaze L. 2008*a*. An auxin transport-based model of root branching in *Arabidopsis thaliana*. *PLoS One* **3,** e3673.

Marhavý P, Vanstraelen M, De Rybel B, Zhaojun D, Bennett MJ, Beeckman T, Benková E. 2013. Auxin reflux between the endodermis and pericycle promotes lateral root initiation. *EMBO Journal* **32,** 149–158.

Meng L, Buchanan BB, Feldman LJ, Luan S. 2012. CLE-like (CLEL) peptides control the pattern of root growth and lateral root development in Arabidopsis. *Proceedings of the National Academy of Sciences, USA* **109,** 1760–1765.

Mishra BS, Singh M, Aggrawal P, Laxmi A. 2009. Glucose and auxin signaling interaction in controlling *Arabidopsis thaliana* seedlings root growth and development. *PLoS One* **4,** e4502.

Moreno-Risueno MA, Van Norman JM, Moreno A, Zhang J, Ahnert SE, Benfey PN. 2010. Oscillating gene expression determines competence for periodic Arabidopsis root branching. *Science* **329,** 1306–1311.

Niu YF, Chai RS, Jin GL, Wang H, Tang CX, Zhang YS. 2012. Responses of root architecture development to low phosphorous availability: a review. *Annals of Botany* (in press).

Notaguchi M, Wolf S, Lucas WJ. 2012. Phloem-mobile *Aux/IAA* transcripts target to the root tip and modify root architecture. *Journal of Integrative Plant Biology* **54,** 760–762.

Okumura K, Goh T, Toyokura K, Kasahara H, Takebayashi Y, Mimura T, Kamiya Y, Fukaki H. 2013. GNOM/FEWER ROOTS is required for the establishment of auxin response maximum for Arabidopsis lateral root initiation. *Plant and Cell Physiology* **54,** 406–417.

Okushima Y, Fukaki H, Onoda M, Theologis A, Tasaka M. 2007. ARF7 and ARF19 regulate lateral root formation via direct activation of *LBD/ASL* genes in Arabidopsis. *The Plant Cell* **19,** 118–130.

Oliva M, Dunand C. 2007. Waving and skewing: how gravity and the surface of growth media affect root development in Arabidopsis. *New Phytologist* **176,** 37–43.

Parizot B, Laplaze L, Ricaud L, *et al.* 2008. Diarch symmetry of the vascular bundle in Arabidopsis root encompasses the pericycle and is reflected in distich lateral root initiation. *Plant Physiology* **146,** 140–148.

Parizot B, Roberts I, Raes J, Beeckman T, De Smet I. 2012. In silico analyses of pericycle cell populations reinforce their relation with associated vasculature in Arabidopsis. *Philosophical Transactions of the Royal Society B: Biological Sciences* **367,** 1479–1488.

Parry G, Calderon-Villalobos LI, Prigge M, Peret B, Dharmasiri S, Itoh H, Lechner E, Gray WM, Bennett M, Estelle M. 2009. Complex regulation of the TIR1/AFB family of auxin receptors. *Proceedings of the National Academy of Sciences, USA* **106,** 22540–22545.

Paul A-L, Amalfitano CE, Ferl RJ. 2012. Plant growth strategies are remodeled by spaceflight. *BMC Plant Biology* **12,** 232.

Péret B, Clément M, Nussaume L, Desnos T. 2011. Root developmental adaptation to phosphate starvation: better safe than sorry. *Trends in Plant Science* **16,** 1360–1385.

Pérez-Torres C-A, Lo´pez-Bucio J, Cruz-Ramı´rez A, Ibarra-Laclette E, Dharmasiri S, Estelle M and Herrera-Estrella L. 2008. Phosphate availability alters lateral root development in Arabidopsis by modulating auxin sensitivity via a mechanism involving the TIR1 auxin receptor. *The Plant Cell* **20,** 3258–3272.

Petersson SV, Johansson AI, Kowalczyk M, Makoveychuk A, Wang JY, Moritz T, Grebe M, Benfey PN, Sandberg G, Ljung K. 2009. An auxin gradient and maximum in the Arabidopsis root apex shown by high-resolution cell-specific analysis of IAA distribution and synthesis. *The Plant Cell* **21,** 1659–1668.

Raya-González J, Pelagio-Flores R, López-Bucio J. 2012. The jasmonate receptor COI1 plays a role in jasmonate-induced lateral root formation and lateral root positioning in *Arabidopsis thaliana*. *Journal of Plant Physiology* **169,** 1348–1358.

Reed RC, Brady SR, Muday GK. 1998. Inhibition of auxin movement from the shoot into the root inhibits lateral root development in Arabidopsis. *Plant Physiology* **118,** 1369–1378.

Richter GL, Monshausen GB, Krol A, Gilroy S. 2009. Mechanical stimuli modulate lateral root organogenesis. *Plant Physiology* **151,** 1855–1866.

Rogg LE, Lasswell J, Bartel B. 2001. A gain-of-function mutation in *IAA28* suppresses lateral root development. *The Plant Cell* **13,** 465–480.

Rowntree, RA, Morris, DA. 1979. Accumulation of ^{14}C from exogenously labeled auxin in lateral root primordia of intact pea seedlings (*Pisum sativum* L.). *Planta* **144,** 463–466.

Roycewicz P, Malamy JE. 2012. Dissecting the effects of nitrate, sucrose and osmotic potential on Arabidopsis root and shoot system growth in laboratory assays. *Philosophical Transactions of the Royal Society B: Biological Sciences* **367,** 1489–14500.

Shkolnik-Inbar D, Bar-Zvi D. 2010. ABI4 mediates abscisic acid and cytokinin inhibition of lateral root formation by reducing polar auxin transport in Arabidopsis. *The Plant Cell* **22,** 3560–3573.

Sun J, Chen Q, Qi L, *et al.* 2011. Jasmonate modulates endocytosis and plasma membrane accumulation of the Arabidopsis PIN2 protein. *New Phytologist* **191,** 360–375.

Sun J, Xu Y, Ye S, *et al.* 2009. Arabidopsis *ASA1* is important for jasmonate-mediated regulation of auxin biosynthesis and transport during lateral root formation. *The Plant Cell* **21,** 1495–1511.

Swarup K, Benková E, Swarup R, *et al.* 2008. The auxin influx carrier LAX3 promotes lateral root emergence. *Nature Cell Biology* **10,** 946–954.

Uehara T, Okushima Y, Mimura T, Tasaka M, Fukaki H. 2008. Domain II mutations in CRANE/IAA18 suppress lateral root formation and affect shoot development in *Arabidopsis thaliana*. *Plant and Cell Physiology* **49,** 1025–1038.

Vidal EA, Araus V, Lu C, Parry G, Green PJ, Coruzzi GM, Gutiérrez RA. 2010. Nitrate-responsive miR393/*AFB3* regulatory module controls root system architecture in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences, USA* **107,** 4477–4482.

Wightman F, Thimann KV. 1980. Hormonal factors controlling the initiation and development of lateral roots. *Physiologia Plantarum* **49,** 13–20.