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Lateral root initiation or the birth of a new meristem

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Abstract

Root branching happens through the formation of new meristems out of a limited number of pericycle cells inside the parent root. As opposed to shoot branching, the study of lateral root formation has been complicated due to its internal nature, and a lot of questions remain unanswered. However, due to the availability of new molecular tools and more complete genomic data in the model species *Arabidopsis*, the probability to find new and crucial elements in the lateral root formation pathway has increased. Increasingly more data are supporting the idea that lateral root founder cells become specified in young root parts before differentiation is accomplished. Next, pericycle founder cells undergo anticlinal asymmetric, divisions followed by an organized cell division pattern resulting in the formation of a new organ. The whole process of cell cycle progression and stimulation of the molecular pathway towards lateral root initiation is triggered by the plant hormone auxin. In this review, we aim to give an overview on the developmental events taking place from the very early specification of founder cells in the pericycle until the first anticlinal divisions by combining the knowledge originating from classical physiology studies with new insights from genetic-molecular analyses. Based on the current knowledge derived from recent genetic and developmental studies, we propose here a hypothetical model for LRI.

Abbreviations: AGP, arabinogalactan protein; CDK, cyclin dependent kinase; KRP, Kip related protein; LR, lateral root; LRI, lateral root initiation; RAM, root apical meristem

Introduction

During embryogenesis, both primary root apical and shoot apical meristems are established, guaranteeing the tender growth of the seedling soon after germination in the subterranean as well as aerial environment. The success of the further outgrowth of the seedling mainly depends on the way root and shoot become capable of exploring the environment. For this purpose, roots and shoots branch intensively. Lateral branches in the shoot develop from axillary buds, which are derived from axillary meristems composed of cells of the shoot apical meristem that never underwent cell enlargement or vacuolation and, therefore, are often regarded as detached meristems (Steeves and Sussex, 1989). In this respect, a lateral branch in the shoot has a clear developmental continuity with the shoot apical meristem and its outgrowth involves the activation of an existing meristem rather than *de novo* conception of a new meristem.

At first sight, root branching differs fundamentally from shoot branching, because no lateral appendices and thus no axillary meristems or axillary buds are formed at the root apical meristem (RAM). In contrast, lateral roots (LRs)

develop from a small group of pericycle cells (or endodermis cells in ferns) situated deep inside the parent root that are not associated with lateral organs. The first visible events of LR formation seem to occur at some distance from the growing tip in the more mature part of the root (Casimiro *et al*., 2001). Therefore, the pericycle cells involved were originally considered as differentiated at the moment of LR initiation (LRI). This characteristic has produced the general concept that dedifferentiation and renewal of cell division is required for LRI. However, by using *Arabidopsis thaliana* (L.) Heynh. as model system, new studies have found evidence that the founder cells (those pericycle cells that will initiate primordia) continue or stay at least competent to divide after leaving the RAM (Dubrovsky *et al*., 2000; Beeckman *et al*., 2001), implying that dedifferentiation is not very common and root and shoot branching might not differ fundamentally.

Another intriguing aspect of LR formation is that only a limited number of pericycle cells become specified as founder cells in a well-defined spatial order. Although the elements involved in founder cell specification are far from clarified, LRI is unambiguously susceptible to a fine-tuned endogenous control mechanism. LRs are regularly spaced along the main root and neatly arranged on longitudinal rows according to the internal vascular construction (Charlton, 1996, and references therein).

Once specified and activated, the founder cells undergo series of precisely oriented cell divisions, giving rise to a LR primordium that will grow through the cortex of the parent root. Based on the division patterns involved, several developmental stages have been defined and differentiation of the different cell types seems to begin already at the earliest stages (Malamy and Benfey, 1997). Finally, the primordium will reach the root surface, penetrate the epidermis, and start its life into the soil environment. At the moment of emergence a highly organized structure with differentiated cells can be observed and a fully functional meristem is established. Because of the endogenous origin of LRs, the study of their formation has been problematic and a lot of questions remain to be answered.

Here, we aim at giving an overview on the developmental events that take place from the very early specification of founder cells in the pericycle until the birth of the new meristem by combining knowledge originating from classical physiology studies and some new insights from genetic-molecular analyses.

How is founder cell identity determined?

The first control point in LR formation is characterized by the specification of founder cells in the pericycle that will give rise to a LR primordium. Unfortunately, this specification is imperceptible and, to date, no marker genes are available that could facilitate the study of this crucial event. Thus, how, when, and where the founder cell identity is laid down is unknown. Based on size and placement of the LR primordia, it can be deduced that in most species founder cell specification happens in pericycle cells at the xylem poles. According to Dubrovsky *et al*. (2000), approximately 11.3% of the divisions in the protoxylem pericycle result in founder cell formation and, hence, establish a lineage leading to LR formation. Until now, there is no consensus on the number of founder cells that contribute to LRI. The average number has been estimated in several ways in various species and no unique basic number has been proposed: 24 (Davidson, 1965) or 162 (MacLeod and Thompson, 1979) for *Vicia faba*, 30 for *Raphanus sativus* (Blakely *et al*., 1982), around 11 (Laskowski *et al*., 1995) or a minimum of 3 (Dubrovsky *et al*., 2001) for *Arabidopsis*. No mutants have been reported with differences in founder cell specification and it is not known if variation in the number of founder cells may affect the size or shape of LRs, as is the case for leaves (Byrne *et al*., 2001).

While there is no clarity on the number, even less is known on the site in the root where founder cells become specified. The first morphological and visible events that follow founder cell specification are transverse and asymmetric divisions that occur in most species far from the root apex in a fully differentiated region, eventually leading to the conclusion that founder cell specification also takes place in this part of the root. However, under certain experimental conditions, LRI could be induced very near the root tip (Gladish and Rost, 1993; Baum *et al*., 1998). Some species initiate spontaneously LR primordia in the meristem itself (Mallory *et al*., 1970). In the

latter study the authors were unable to make the distinction between the proliferative divisions of the meristem and those involved in early primordium formation.

Therefore, founder cell identity is most likely specified in the RAM itself or at least in close proximity of the tip. Whether the mechanisms involved in the radial patterning of the meristem itself play a role in this process is not clear. Normal LRI still goes on in the *hydra* mutants that present a defective radial pattern with supernumerous cell layers and aberrant vascular patterning in the root tip (Souter *et al*., 2004). Apparently, in such roots with seriously disorganized RAMs, normal specification of LR founder cells can still proceed.

The pericycle as an 'extended meristem'

The specification of LR founder cells in the root apex does not at all concur with the generally accepted idea that LRs are initiated by the dedifferentiation of pericycle cells distal to the meristem. Indeed, LRI has since long been considered to occur after re-entry of pericycle cells into the cell cycle from an arrested G2 phase (Blakely and Evans, 1979; Laskowski *et al*., 1995; Malamy and Benfey, 1997).

However, during recent years, experimental evidence is accumulating that argues against this dedifferentiation concept. In maize seedlings, the time interval between meristem exit and LRI by pericycle cells was found to be relatively short (Dubrovsky and Ivanov, 1984), leaving little, if any, time for differentiation. Furthermore, in *Arabidopsis* and *Medicago* sp., the pericycle has been shown to have constitutive potency to divide, because of the constitutive expression of at least two core cell cycle genes, the cyclin-dependent kinase *CDKA;1* and the cyclin *CYCA2;1* (Beeckman *et al*., 2001; Roudier *et al*., 2003). Furthermore, pericycle cells have been observed that actually continue to divide at either the xylem (*Arabidopsis thaliana*, *Pisum sativum*, and *Allium cepa*) or phloem poles (*Daucus carota*), depending on the site of LRI (Lloret *et al*., 1989; Dubrovsky *et al*., 2000). However, most of these divisions do not result in LRI, and are purely proliferative. Accordingly, based on the genetic and phenotypic characterization of the *alf4-1* mutant (see also below), DiDonato *et al*. (2004) have found evidence that the pericycle shares properties with meristems, i.e. the ability to continue cell division. It was proposed that the ALF4 protein keeps the pericycle in an indispensable mitosis-competent state, because the *alf4-1* mutant failed to express *CYCB1;1::GUS* in the pericycle. The pericycle would then play a central role in creating the developmental plasticity needed for root system development. Even at the cytological level, pericycle cells at the xylem poles seem to exhibit constitutively meristematic features, such as large nuclei, small vacuoles and dense cytoplasm (Himanen *et al*., 2004).

In conclusion, the protoxylem pole pericycle cells most probably do not have to dedifferentiate prior to LRI, but seem to be susceptible to rapid division. Based on their undifferentiated nature, the pericycle cells beyond the RAM could be called an 'extended meristem', as proposed earlier by Casimiro *et al*. (2003).

The first division of the founder cells or lateral root initiation

After specification, pericycle founder cells proceed to the second hallmark of LR formation, namely the first formative division, which is generally equated with LRI itself, because it represents the first visible event. The orientation of these divisions is chiefly transversal and anticlinal, immediately followed by periclinal divisions. In earlier literature, usually anticlinal and periclinal divisions were (or could) not be distinguished with regard to LRI (Esau, 1965; Fahn, 1974). Yet, evidence is abundant that a series of anticlinal divisions precede periclinal divisions (Casimiro *et al*., 2003).

Cell divisions that give rise to daughter cells with various fates are essential to generate the multitude of different cell types present in multicellular organisms. In plants, the control of the cell division plane has traditionally been considered important for the formation of regular patterns. The onset of several developmental programs in plants, such as embryogenesis and stomata formation, coincides with asymmetric divisions.

Clearly, during LRI, the first anticlinal divisions are asymmetric and divide the pericycle founder cells in unequally sized daughter cells. Asymmetric division is probably the most

common type of division at the onset of LRI, because it has been observed in nearly all analyzed species so far: *Allium cepa* (Casero *et al*., 1993), *Zea mays* (Bell and McCully, 1970; Ashford and McCully, 1973; Casero *et al*., 1995), *Raphanus sativus* (Blakely *et al*., 1982), *Lactuca sativa* (Zhang and Hasenstein, 1999), and *Oryza sativa* (Kawata and Shibayama, 1965). Also, in *Arabidopsis*, it could be demonstrated that longitudinally adjacent pericycle cells divide in such a manner that two shorter cells are produced next to each other (Casimiro *et al*., 2001; Figure 1A). These two small daughter cells represent the center of a future primordium. This type of LRI was designated 'longitudinal bicellular' (Dubrovsky *et al*., 2001). However, Dubrovsky *et al*. (2001) also described a 'longitudinal unicellular' initiation, when only one pericycle cell becomes a founder cell for the entire longitudinal extent of the LR primordium. Nonetheless, this type of initiation appears to be less common.

In *Arabidopsis*, the study of LRI is facilitated through markers, such as the mitotic cyclin *CYCB1;1*::*GUS* for the first anticlinal division (Beeckman *et al*., 2001) and *DR5::GUS* for the auxin response (Benková *et al*., 2003). How the positioning of the division plane is regulated during LRI is still unclear. It is assumed that the asymmetric division is preceded by a nuclear migration, because nuclei displaced towards the neighboring cell can be observed on longitudinal sections during asymmetrical transverse divisions (Casero *et al*., 1993). However, this nuclear movement has not yet been proven *in vivo*.

In summary, within pericycle founder cells, the nuclei are thought to move towards one end, followed by an anticlinal asymmetric division of the central smaller cell. The newly divided cells are apparently susceptible to repolarizing stimuli, because the asymmetric divisions result in the formation of a new organ with a polarity perpendicular to that of the original parent root.

The site of lateral root initiation

In most species, the origin of LR primordia is distal to the main root apex in the differentiation

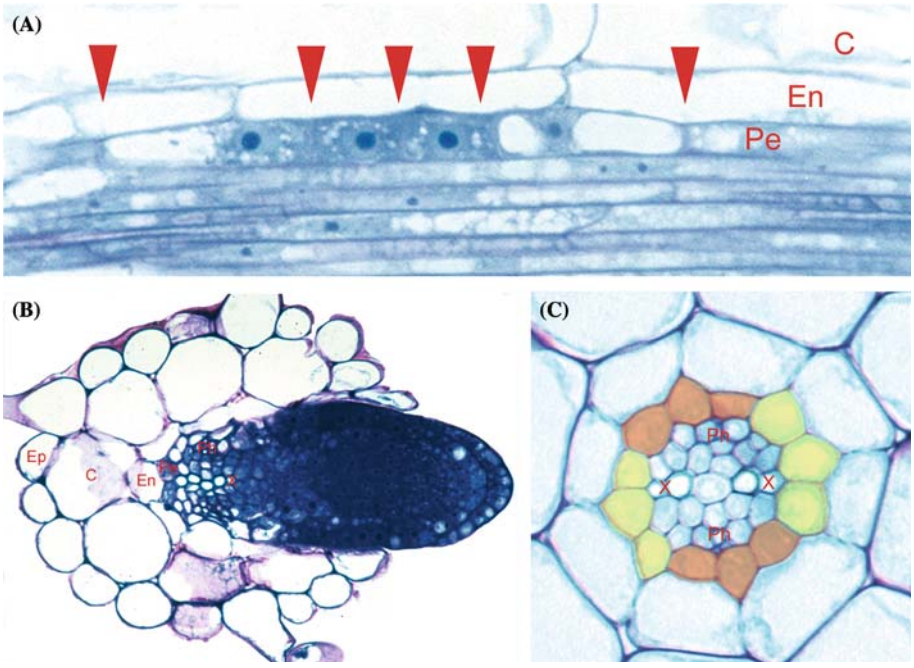

Figure 1. Morphological and anatomical characteristics of the pericycle and LRI in *Arabidopsis*. (A) The first visible manifestation of LRI, i.e. the alignment of two short cells in the midst of two larger cells (cell walls are indicated with arrowheads). (B) Transverse section through an emerging LR opposite the xylem pole. (C) Two distinct pericycle cell populations can be recognized, one opposite the xylem pole (yellow) and one opposite the phloem pole (orange). Symbols: C, cortex; En, endodermis; Ep, epidermis; Pe, pericycle; Ph, phloem; X, xylem.

zone, which seems to coincide with the appearance of the first vascular elements, i.e. the protophloem (Mallory *et al*., 1970), or the differentiating or mature protoxylem (Charlton, 1996). However, this spatial relationship with vascular tissue differentiation is not a prerequisite for LRI, because in a few species, such as *Marsilea*, *Ceratopteris*, *Eichornia*, *Pistia*, *Musa*, *Cucurbita* (Dubrovsky and Rost, 2003), and *Cladopus javanicus* (Koi and Kato, 2003), LRI occurs within the RAM, where no vascular tissue has differentiated yet.

The site of LRI appears to be controlled by the RAM or at least a factor derived from it. The removal of the root apex in various species could stimulate LRI primarily in the region behind the cutting site (McCully, 1975), which might probably be the result of the removal of an inhibitory factor originating from the RAM. A few reports suggested that the phytohormone cytokinin would play this role during LRI (Torrey, 1962, Blakely *et al*., 1972).

The most remarkable and least understood aspect related to the LRI site is the circumferential spacing around the parent root axis, hinting at a tight relationship with the internal vascular structure. Indeed, in most species, the site of primordia development is exclusively restricted to pericycle cells opposite the xylem pole (e.g. *Lactuca sativa* cv. Baijianye [Zhang and Hasenstein, 1999], *Raphanus sativus* [Casero *et al*., 1995], *Helianthus annuus* [Casero *et al*., 1995], *Pisum sativum* [Lloret *et al*., 1989], and *Arabidopsis thaliana* [Dubrovsky *et al*., 2001]) (Figure 1B). However, several exceptions have been reported that form LRs at the phloem poles, such as *Daucus carota*, *Zea mays* (Casero *et al*., 1995), *Triticum vulgare* (Foard *et al*., 1965), and *Oryza sativa* (Nishimura and Maeda, 1982). It is striking that these exceptions appear to belong mainly to monocotyledonous species.

It is not clear what determines the specificity for xylem or phloem pole, but this pattern also occurs in species that initiate LRs in the endodermis. In *Marsilea quadrifolia* (Lin and Raghavan, 1991) and the fern *Ceratopteris richardii* (Hou *et al*., 2004), LRs arise from endodermis cells located opposite the protoxylem poles within the meristematic region of the parent root.

Obviously, the position of LRs in the vicinity of a xylem pole must have an important developmental advantage. Other processes also favor the xylem pole as initiation site. Root nodule formation (Heidstra *et al*., 1997) and nematode infection (Golinowski *et al*., 1997) are strongly correlated with the xylem. It can be assumed that a direct contact with that part of the vascular transport system might be beneficial for these processes, because xylem is responsible for the root-shoot transport of water and dissolved ions. Even when the LR is initiated in front of the phloem, as is the case for maize, the developing primordia encompass two xylem strands later on (Bell and McCully, 1970), which might enhance the contact even more. Another more mechanical interpretation attributes a protecting function to the xylem in offering resistance against the pressure exerted upon the stele by the growing primordium (Barlow *et al*., 2004).

The heterogeneous nature of the pericycle

As mentioned above, LRs are specifically initiated at either the xylem or phloem pole. Several detailed studies on the pericycle cell layer in various species have suggested the existence of two different cell types even before any sign of LRI. In roots of *Daucus carota*, a species with phloem pole-specific LRI, the pericycle is uniseriate opposite the phloem and has two layers of cells in front of the xylem pole (Casero *et al*., 1998).

In several species, the cells in front of the xylem pole are shorter than those in front of the phloem (Luxová, 1975; Blakely *et al*., 1982; Casero *et al*., 1989a; Lloret *et al*., 1989; Dubrovsky *et al*., 2000). Also pericycle cells at the xylem pole are more radially expanded than those in front of the phloem pole (Laskowski *et al*., 1995). In addition, some cytological features, i.e. the presence of spherical granules in the cytoplasm in a part of the pericycle cells and different affinities for methyl blue staining, have been described, allowing the recognition of alternating populations of cells in the pericycle at a very early stage of differentiation (Toriyama 1978a, 1978b). Additionally, structural heterogeneities at the level of the cell surface of pericycle cells have been observed. Using monoclonal antibodies for arabinogalactan proteins (AGPs) and extensins, Knox *et al*. (1989) and Casero *et al*. (1998) have shown radial differences within the pericycle of several species. In carrot, AGPs have been specifically localized at the xylem

pericycle and extensins in pericycle cells at the phloem pole, whereas in pea, pericycle cells labeled with anti-AGP have been found at the phloem poles and with anti-extensin at the xylem pole. AGPs and extensins might be essential in the coordination of cell proliferation and cell expansion, making them likely candidates to regulate the cell diameter and meristematic status of the xylem pole pericycle cells as opposed to those at the phloem pole.

Also at the level of the distribution of plasmodesmata, phloem and xylem pole pericycle cells differ. Although the entire pericycle seems to be radially interconnected through plasmodesmata, numerous plasmodesmatal connections between the pericycle and the metaphloem companion cells have been observed, while this was less apparent between the pericycle and xylem tissues (Wright and Oparka, 1997; Complainville *et al*., 2003).

In contrast to other cells generated by the RAM, in *Arabidopsis*, pericycle cells adjacent to the protoxylem poles of the vascular cylinder continue to cycle without interruption during their passage through the elongation and differentiation zones (Dubrovsky *et al*., 2000; see above). The files of shorter pericycle cells maintain a proliferative activity for a greater period than the longer cells (Rost *et al*., 1988; Casero *et al*., 1989b; Dubrovsky *et al*., 2000). However, only some of the dividing pericycle cells are committed to the asymmetric, formative divisions that give rise to LR primordia (Dubrovsky *et al*., 2000).

The above examples illustrate that the pericycle is not at all homogenous and that clear differences between the cells at either phloem or xylem pole allow us to distinguish two populations within the pericycle tissue layer (Figure 1C). However, it is unclear what comes first. Are the differences within the pericycle a demonstration of the potency for LRI or does potency for LRI give rise to these differences?

Auxin and the cell cycle at the onset of lateral root initiation

So far, we have focused mainly on the structural and cytological peculiarities of LRI. Plants tightly control LRI because LRs are initiated at well-defined positions (see above); but, through which mechanism this happens is not yet fully understood. Nevertheless, it is obvious that auxin plays a dominant role in this process. The application (Torrey, 1950; Himanen *et al*., 2002) and endogenous overproduction (Boerjan *et al*., 1995; Celenza *et al*., 1995; King *et al*., 1995) of auxin result in an increase in the LR number. More recently, in-depth analyses have strongly confirmed the role of auxin during LRI. Firstly, Benková *et al*. (2003) have shown the presence of auxin and/or auxin response just prior to and during the asymmetric division with an auxin-responsive promoter, *DR5::GUS*. Secondly, several other auxin-related genes are expressed during LRI (Marchant *et al*., 2002; Tatematsu *et al*., 2004). Thirdly, when auxin transport is inhibited, LRI is also prevented (Casimiro *et al*., 2001).

So, auxin is clearly required for the initial cell divisions during LRI. How auxin interacts with the cell cycle is the central theme of a review by Vanneste *et al*. (2005). Here, we will briefly summarize the most striking new findings concerning this interaction.

Recently, Himanen *et al*. (2002) have demonstrated that auxin, while triggering LRI, connects to the cell cycle at the G1-to-S transition. This specific interaction might coincide or even be crucial for the determination of founder cell identity. Although the majority of the different components of the basic cell cycle machinery in plants has been identified (De Veylder *et al*., 2003), it is not unambiguously clear which cell cycle genes are primarily involved during LRI. One widely accepted aspect is the high level of cell division competence of the xylem pole pericycle cells, as illustrated by the expression of the *CDKA;1* gene (Beeckman *et al*., 2001). It would be logic to conclude that during LRI, auxin would directly activate this *CDKA;1* with the anticlinal divisions as a consequence. However, the activity of CDKs is controlled at various levels such as phosphorylation, interaction with inhibitory and docking proteins, and, last but not least, binding of a cyclin-regulatory subunit. Therefore, cyclins are the most patent factors to think of while searching for auxin-induced CDK activity. Interestingly, in mammals, the G1-to-S transition is initiated by the *de novo* synthesis of D-type cyclins (Weinberg, 1995) and D-type cyclin levels in *Arabidopsis* cell suspensions are highly responsive to the addition of auxin (Fuerst *et al*., 1996; Richard *et al*., 2002). On the other hand, even at the CDK expression level itself,

auxin might induce cell division because the plant-specific *CDKB1;1* seems to be induced by auxin (Richard *et al*., 2002; Himanen *et al*., 2002). B-type cyclins genes, such as *CYCB1;1*, expressed at the initiation sites (Beeckman *et al*., 2001) might not be primarily involved in the auxin-mediated cell cycle activation; their expression is most probably the consequence of reactivated cell division, which would explain that ectopic expression of *CYCB1;1* driven by the *CDKA;1* promoter does not result in enhanced LRI (Doerner *et al*., 1996). Besides these putative positive regulators, a negative control, exerted by CDK inhibitors or Kip-related proteins (KRPs) plays a crucial, albeit less clear, role. The *KRP2* gene is downregulated upon auxin treatment and, when overexpressed, strongly reduces the LR number (Himanen *et al*., 2002). This gene is strongly expressed in those regions where no LRI occurs, i.e. in pericycle cells opposite a LRI site and at the phloem poles, both in *Arabidopsis* and radish (Himanen *et al*., 2002). However, until now, no promoting effect upon LRI through a direct interference with cell cycle regulation has been detected.

The genetic analysis of lateral root initiation

Several approaches have been undertaken to elucidate the genetic control of LRI, but genes directly controlling LRI have not been identified yet. We will give an overview of the outcome of genetic research that uses mainly *Arabidopsis* as model species.

Auxin plays a dominant role in LRI (see above) and this characteristic feature has been successfully exploited in several mutant screenings. As a result, several mutants have been identified in auxin signaling (*axr* mutants, *tir1*, *msg2-1*, *shy2*), auxin transport (*aux1* and *pin* mutants), and auxin homeostasis (*alf1*, *ydk1*, *dfl1*) with a distinct LR phenotype (see Table 1, and references therein). Because it is impossible to focus on all of the identified mutants in the framework of this review, we will restrict ourselves to those genes that, based on the mutant phenotypes, might be directly involved in LRI, more precisely the asymmetric division that initiates a LR. It should be emphasized that LRI does not necessarily have to be affected in mutants with a reduction in clearly visible, outgrown LRs. A detailed examination of the occurrence or absence of asymmetric divisions in the xylem pole pericycle is required before a mutant can be categorized as LRI mutant. Such analyses lack unfortunately in most of the mutants described.

Most of the LR mutants (approximately 40% of those listed in Table 1) are affected in a specific part of the auxin pathway and their phenotypes can usually be rescued or mimicked through auxin application (Boerjan *et al*., 1995; Marchant *et al*., 2002). In addition to the well-known auxin mutants, several other mutants have also been described with a defect in LRI. A more or less equal number of mutants have a reduced or increased number of LRs (Table 1).

Only two *Arabidopsis* mutants, *slr-1* (Fukaki *et al*., 2002) and *alf4-1* (Celenza *et al*., 1995), are not capable of initiating any LRs. In the *slr-1* mutant, the AUX/IAA protein IAA14 is stabilized, strongly inhibiting the auxin-signaling pathway towards LRI, with a nearly complete inhibition of the anticlinal division in the pericycle and a complete block of the periclinal divisions in the divided pericycle cells (Fukaki *et al*., 2002).

Recently, DiDonato *et al*. (2004) have shown that ALF4 functions independently from auxin signaling but is instead required to maintain the pericycle in a mitosis-competent state needed for LR formation. The competent state appears to be a prerequisite for the very first asymmetric divisions, because no such divisions and no *CYCB1;1* expression are observed in the mutant. Curiously, the expression of *CDKB1;1::GUS* increases in the *alf4-1* pericycle, which might suggest a delay or block of the cell cycle soon after DNA synthesis and prior to the onset of *CYCB1;1* expression. Secondly, it puts forward the idea that auxin-independent control mechanisms on cell cycle regulation are operating just before the actual first divisions and that the above described auxin-induced G1-to-S transition is only part of the story.

On the other hand, all induced cell cycle activation in pericycle cells does not necessarily have to result in LRI. In weak *gnom* alleles, the whole pericycle shows a homogeneous proliferation when stimulated with auxin; however, the mutants are not able to specifically trigger LRI (Geldner *et al*., 2004). It is hypothesized that GNOM would be a critical factor in determining the polarity of the daughter cells through interference with the localization of auxin efflux carriers (PINs). This localization would canalize and

accumulate auxin in some cells of the pericycle, which, in turn, would proliferate and deplete auxin from the adjacent cells, thus, inhibiting their proliferation. This GNOM/PIN-mediated auxin canalization might be the crucial factor in determining founder cell identity and/or the driving force for the asymmetric divisions.

Most of our knowledge on the genetic control on LR formation has been acquired from research on dicotyledonous species. In monocots, only a small number of mutants related to root formation have been found (Table 1) and only one maize mutant, *lrt1*, lacks LRs (Hochholdinger and Feix, 1998). Interestingly, although all different types of early postembryonic roots are completely devoid of LRs in this mutant, the later formed crown roots on the stem form LRs, indicating the presence of at least two root-type or phase-specific pathways for LRI in maize and maybe also in other monocots. The genes corresponding to these mutants have not been cloned yet, but these studies are useful to understand the mechanism of root development in monocots that might be at some point fundamentally different from that in dicots.

Besides the dominant role of auxin, several other hormones (cytokinins and abscisic acid) and nutrients (nitrate, phosphate, and sugar) affect LR formation (Table 1). As mentioned earlier LR formation is not restricted to LRI (Malamy and Benfey, 1997), so LR phenotypes can arise from impeding other developmental stages. A few genes involved in LR emergence have been described (Hunter *et al*., 2003; Neuteboom *et al*., 2003). De Smet *et al*. (2003) have shown that abscisic acid prevents primordium outgrowth just after emergence through an arrest in meristem activation. Changes in nitrate and phosphate availability have been found to affect LR formation and elongation (Zhang and Forde, 1998; Williamson *et al*., 2001; Linkohr *et al*., 2002). A mutation in one of the genes that controls the above mentioned pathways and stages might result in altered root architecture (Table 1), although they are not necessarily involved in LRI.

Expression analysis of lateral root initiation

Several genes have been described that are expressed in the pericycle and/or during early LRI in several plant species (Keller and Lamb, 1989; Wyatt *et al*., 1993; Taylor and Scheuring, 1994; Williams and Sussex, 1995; Liu and Ekramoddoullah, 2003; Tan *et al*., 2003). However, such small-scale expression analyses did not result in the identification of important positive regulators of LRI.

In addition to the 'one-gene' approaches, few studies have been undertaken to analyze LRI on a larger scale, by using cDNA substraction or microarray analysis (Sussex *et al*., 1995; Neuteboom *et al*., 1999; Himanen *et al*., 2004). All of the approaches are again based on the knowledge that auxin triggers LRI. Sussex *et al*. (1995), who analyzed gene expression patterns in newly organizing radish LRs on the basis of two subtracted cDNA libraries enriched for genes that are expressed at specific times and stages in LR development after auxin application, mainly identified ribosomal proteins. Neuteboom *et al*. (1999) isolated and characterized cDNA clones corresponding with mRNAs that accumulate during auxin-induced LR formation, e.g. AIR1, AIR3, AIR9 and AIR12. Although most probably involved at some point during LR formation, these proteins appeared not to be crucial regulators for LRI. For instance, AIR3 (a protein that possesses all the characteristics of subtilisin-like proteases) might weaken cell-to-cell connections and thus facilitate LR emergence (Neuteboom *et al*., 1999).

The genes identified in the above two approaches obviously do not play a key regulatory role but are required in starting up the basic machinery during LRI or are involved at later stages of LR development. To identify important genes involved in the actual LRI process, a more targeted approach is required. Recently, Himanen *et al*. (2004) performed a broad-scale transcript profiling using a LR-inducible system, in which most xylem pole pericycle cells were synchronously activated by auxin transport inhibition followed by auxin application (Himanen *et al*., 2002). Of 4600 genes analyzed, 906 significantly differentially regulated genes were identified. Most of the key aspects in the cascade towards LRI were present in this dataset, which includes genes involved in auxin transport and signaling (*LAX3*, *IAA2*, and *IAA11*), G1-to-S transition (*E2Fa* and *Histone H4*) and G2-to-M transition (*CYCB1;1*, *CYCB2;1*, and *CDKB1;1*). This transcript profiling study is the first report that reflects the transcriptional changes during root

Table 1. List of genes affecting LR initiation and/or development.

The model species *Arabidopsis thaliana*				
Gene	AGI	Mutant/transgene	LR phenotype	Publication
Auxin signaling/homeostasis/transport				
Classical auxin signalling cascade				
AXR1	AT1G05180	*axr1-3*	Reduced LR number	Lincoln *et al.* (1990)
		axr1-12	Reduced LR number	
CUL1	AT4G02570	*axr6-1 / axr6-2*	Reduced LR number	Hobbie *et al.*, (2000)
TIR1	AT3G62980	*tir1-1*	Reduced LR number	Ruegger *et al.* (1998)
IAA1	AT4G14560	*axr5-1*	Reduced LR number (on auxin)	Yang *et al.* (2004)
IAA3	AT1G04240	*shy2-2*	Reduced LR number	Tian and Reed (1999)
		shy2-22 (loss of function allele)	Increased LR number	
		shy 2-24 (loss of function allele)	Increased LR number	
IAA14	AT4G14550	*slr-1*	Reduced LR number (lack LRs)	Fukaki *et al.* (2002)
IAA19	AT3G15540	*msg2-1*	Reduced LR number	Tatematsu *et al.* (2004)
IAA28	AT5G25890	*iaa28-1*	Reduced LR number	Rogg *et al.* (2001)
NAC1	AT1G56010	Antisense *35S::NAC1*	Reduced LR number	Xie *et al.* (2000)
		Overexpression *35S::NAC1*	Increased LR number	
SINAT5	AT4G27880	Overexpression *35S::SINAT5*	Reduced LR number	Xie *et al.* (2002)
		Dominant negative *35S::SINAT5(C49S)*	Increased LR number	
Auxin transport				
AUX1	AT2G38120	*aux1* alleles	Reduced LR number	Marchant *et al.* (2002) and Maher and Martindale (1980)
PINs: *PIN1*	AT1G73590	Overexpression *35S::PIN1*	Retarded primordia growth	Benková *et al.* (2003)
PIN3	AT1G70940	*pin1 pin3*	Reduced LR number/slower development	
PIN4	AT2G01420	*pin3 pin7, pin4 pin7, pin1 pin4 pin7, pin1 pin3 pin7*	Development of less well-defined primordia	
PIN7	AT1G23080	*pin1 pin3 pin4*	Massive division of pericycle cells without defined primordia	
TIR3	AT3G02260	*tir3-1*	Reduced LR number	Ruegger *et al.* (1997)
		asa1 / umb1	Reduced LR number	Kanyuka *et al.* (2003)
PINOID	AT2G34650	Overexpression *35S::PID*	Reduced LR number	Christensen *et al.* (2000) and Benjamins *et al.* (2001)
GNOM	AT1G13980	Weak *gnom* alleles	Reduced LR number (failure in initiation)	Geldner *et al.* (2004)
Auxin homeostasis				
ALF1	AT2G20610	*alf1/sur1/rty*	Increased root number	Boerjan *et al.* (1995), Celenza *et al.* (1995) and King *et al.* (1995)
SUR2	AT4G31500	*sur2/rnt1*	Increased LR number	Barlier *et al.* (2000), Delarue *et al.* (1998) and Bak *et al.* (2001)

Table 1. Continued.

The model species *Arabidopsis thaliana*				
Gene	AGI	Mutant/transgene	LR phenotype	Publication
ILR2	AT3G18485	*ilr2-1*	Reduced LR number	Magidin *et al.* (2003)
ARF8	AT5G37020	*arf8-1*	Increased LR number	Tian *et al.* (2004)
		Overexpression *35S::ARF8*	Reduced LR number	
ILR1	AT3G02875	*ilr1 iar3 ill2*	Reduced LR number	Rampey *et al.* (2004)
IAR3	AT1G51760			
ILL2	AT5G56660			
YDK1	AT4G37390	*ydk1-D*	Reduced LR number	Takase *et al.* (2004)
		Overexpression 35S::*YDK1*	Reduced LR number	
DFL1	AT5G54510	*dfl1-D*	Reduced LR number	Nakazawa *et al.* (2001)
		antisense *35S::DFL1as*	Increased LR number	
		Sense *35S::DFL1*	Reduced LR number	
Effects on LR number and development				
Other genes affecting the LR number				
ALF4	AT5G11030	*alf4-1*	Reduced LR number (lack LRs)	DiDonato *et al.* (2004) and Celenza *et al.* (1995)
AXR4	?	*axr4-1* / *axr4-2*	Reduced LR number	Hobbie and Estelle (1995)
		axr4-1 axr1-3	Stronger reduction in LR number	
ATHB-2	AT4G16780	Overexpression 35S::*ATHB-2*	Reduced LR formation	Steindler *et al.* (1999)
		Reverse ATHB-2 sequence 35S:: α*ATHB-2*	Increased LR formation	
ATHB-8	AT4G32880	Overexpression	Reduced LR formation	Baima *et al.* (2001)
ERA1	AT5G40280	*era1-2*	Increased LR number	Brady *et al.* (2003)
ETA3	AT4G11260	*eta3*	Reduced LR number	Gray *et al.* (2003)
GPA1	AT2G26300	*gpa1-1* / *gpa1-2*	Reduced LR number	Ullah *et al.* (2003)
AGB1	AT4G34460	*agb1-1* / *agb1-2*	Increased LR number	Ullah *et al.* (2003)
HAT2	AT5G47370	Overexpression	Reduced LR elongation	Sawa *et al.* (2002)
HY5	AT5G11260	*hy5-1* / *hy5-Ks50*	Increased LR number	Oyama *et al.* (1997)
PXA1	AT4G39850	*pxa1*	Reduced LR number	Zolman *et al.* (2001)
RAV1	AT1G13260	Overexpression	Retarded LR development	Hu *et al.* (2004)
RCN1	AT1G25490	*rcn1*	LR growth less NPA sensitive	Rashotte *et al.* (2001)
RIB1	?	*rib1*	Increased LR number	Poupart and Waddell (2000)
ROP2	AT1G20090	*CA-rop2* (constitutively activate)	Increased LR number	Li *et al.* (2001)
		DN-rop2 (block)	Reduced LR number	
SBR	?	*sbr*	Reduced LR number	Subramanian *et al.* (2002)
KNAT6	AT1G23380	*35S::RNAi*	Increased LR number	Dean *et al.* (2004)
MRP5	AT1G04120	*mrp5-1*	Increased LR number	Gaedeke *et al.* (2001)
SEU	AT1G43850	*seu-3*	Reduced LR number	Pfluger and Zambryski (2004)
XPL1	?	*xipotl*	High number of LRs	Cruz-Ramirez *et al.* (2004)
RML2	?	*rml2*	No LRs	Cheng *et al.* (1995)

PLT1 and PLT2		?	*plt1 plt2*	Increased LR number	Aida *et al.* (2004)
XBAT32		?	*xbat32-1*	Reduced LR number	Nodzon *et al.* (2004)
Cytokinin related genes					
A type ARRs:	*ARR3*	AT1G59940	type-A *arr* mutations and higher order mutants	Reduced LR formation	To *et al.* (2004)
	ARR4	AT1G10470			
	ARR5	AT3G48100			
	ARR6	AT5G62920			
	ARR8	AT2G41310			
	ARR9	AT3G57040			
CKX genes:	*CKX1*	?	Overexpression *35S::CKX1*	Increased LR number	Werner *et al.* (2003)
	CKX3	AT5G56970	Overexpression *35S::CKX3*	Increased LR number	
PAS1		AT3G54010	*pas1*	Reduced LR number	Faure *et al.* (1998)
PAS2		AT5G10480	*pas2*	Increased LR number	Faure *et al.* (1998)
PAS3		?	*pas3*	Reduced LR number	Faure *et al.* (1998)
Genes affecting primordium formation, meristem development/activation, LR outgrowth and elongation					
ALF3		?	*alf3-1*	Arrested LR development	Celenza *et al.* (1995)
RML1		AT4G23100	*rml1*	Arrested LR development	Cheng *et al.* (1995)
HOBBIT		AT2G20000	weak *hbt* allele	LR meristem defect	Willemsen *et al.* (1998)
ABI3		AT3G24650	*abi3-6*	Required for correct auxin response in LRP	Brady *et al.* (2003)
WAK4		AT1G21210	DEX-induced *WAK4 antisense*	Inhibition of LR development (elongation)	Lally *et al.* (2001)
RanBP1c		AT5G58590	Antisense *AtRanBP1c*	Reduced LR number (arrested outgrowth)	Kim *et al.* (2001)
LIN1		?	*lin1*	No LR repression	Malamy and Ryan (2001)

Other species

Gene	Species	Mutant/transgene	LR phenotype	Publication
SEMAPHORE1	*Zea mays*	*sem1-R*	Reduced LR number	Scanlon *et al.* (2002)
LRT1	*Zea mays*	*lrt1*	Reduced LR number (lack LRs)	Hocholdinger and Feix (1998)
SLR1	*Zea mays*	*slr1*	Short LRs (impaired root cell elongation)	Hocholdinger *et al.* (2001)
SLR2	*Zea mays*	*slr2*	Short LRs (impaired root cell elongation)	Hocholdinger *et al.* (2001)
CKI1	*Oryza sativa*	Antisense	Reduced LR number	Liu *et al.* (2003)
ARM1 / ARM2	*Oryza sativa*	*arm1*	Reduced LR number	Chhun *et al.* (2003)
		arm2	Slightly reduced LR number	
		arm1 arm2	Even more reduced LR number	
HAR1	*Lotus japonicus*	*har1-1/har1-2/har1-3*	Bushy root	Krusell *et al.* (2002)
RH2-1	*Medicago sativa*	Overexpression	Reduced LR number	Karlowski and Hirsch (2003)
HR7	*Hyoscyamus niger*	Overexpression	Increased LR formation	Mikami *et al.* (1999)
DGT	*Lycopersicon esculentum*	*diageotropica*	Reduced LR number (lack LRs)	Muday *et al.* (1995)
NAC14	*Brassica napus*	Overexpression	Increased LR number	Hegedus *et al.* (2003)

branching in *Arabidopsis* and that might identify key regulators. Notwithstanding the identification of several core cell cycle genes involved in LRI, this approach still gave an incomplete view, because it was not performed on a genome-wide scale.

More focused genome wide approaches are currently undertaken such as the use of mutants as negative controls (S. Vanneste, unpublished data) or tissue specific approaches (I. De Smet, unpublished data) in broad-scale transcription profiling studies. These strategies will allow us to narrow down the number of potential players in LRI. So, even though expression analyses on a larger scale may result in the identification of several genes involved in LRI, no functional correlation has been successfully demonstrated yet.

Conclusion

Although the importance of (lateral) root research, recently emphasized by Epstein (2004), and the increasing interest of scientists in the hidden half of the plant (Table 1), the key regulatory genes, directly involved in promoting LRI, still have to be identified. All the known mutants, transgenes, and expression patterns taken together, it is clear that the most progress has been made on the aspects of auxin synthesis, polar transport, signal transduction, and degradation that affect LR development but are not direct triggers of LRI. Various genes involved in one of the above processes affecting LR development have been described (Table 1).

Based on the current knowledge derived from genetic and developmental studies described above, we propose a hypothetical model for LRI (Figure 2). Although the precise order, timing, and localization of the several activities leading to LRI are not fully determined, an early and maybe the very first event is the specification of the LR founder cells. How this is established is not known, but it probably takes place in or nearby the RAM. Secondly, the xylem pole pericycle cells enter the S-phase, a process clearly induced by auxin that coincides with an inhibition of cell division at the phloem pole, as illustrated by the KRP2 protein (Himanen *et al*., 2002).

After the DNA synthesis has been completed, ALF4 is required to bring the cells in a mitosis-competent state. Both the G1-to-S transition and the achievement of the mitotic competence controlled by ALF4 appear not to be restricted to a small subset of pericycle cells at the xylem poles. Based on cell cycle gene expression studies

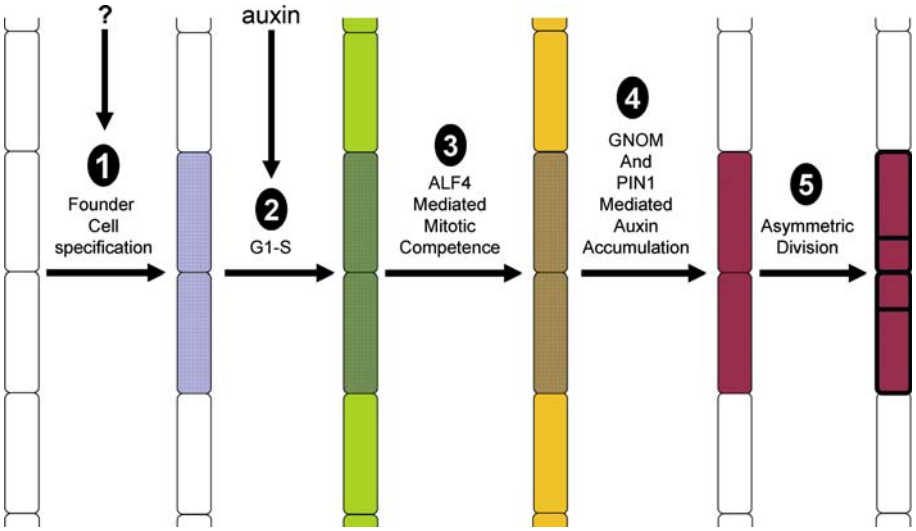

Figure 2. *Hypothetical* model for LRI (for clarity, only one xylem pole pericycle cell file is partly depicted). From left to right, a cascade of possible events is represented, the first event being the specification of the founder cells (1). Up to now, the initial trigger for founder cell specification is unknown (as indicated by '?'). Secondly, part of or the whole cell file undergoes an auxin-induced G1-to-S transition (2), which is followed by a premitotic phase mediated by ALF4 (3). Then, an auxin gradient is built up in the founder cells through the GNOM-mediated targeting of the auxin efflux carrier PIN1 to particular cell walls (4). Finally, the asymmetric division is initiated (5; thick line).

(Beeckman *et al*., 2001; DiDonato *et al*., 2004) and the cytological meristematic properties of the protoxylem pole pericycle cells, it is more than likely that besides the LR founder cells other pericycle cells are also kept in a cell division-susceptible state. This state would explain the capacity of plant roots to respond rapidly to environmental signals by producing new LRs out of the acropetal sequence. Finally, local auxin accumulation in the founder cells is built up via the GNOM/PIN1-controlled auxin efflux (Geldner *et al*., 2004). This auxin accumulation is believed to induce the asymmetric divisions of the founder cells and represents the onset for LR formation. In conclusion, LRI appears to depend on the interplay between auxin, cell cycle progression, cell fate, and the developmental status of the pericycle. When all these elements together are met in a few cells, LRI can proceed.

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