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Developmental Biology of Roots: One Common Pathway for All Angiosperms?

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ABSTRACT

The primary root meristem is formed during embryogenesis and supports the first growth of the seedling into the soil. However, the continuous growth of the above-ground plant parts imposes the establishment of an elaborate root system in order to mine for additional water and nutrients. This can only be achieved by *de novo* installation of extra root meristems soon after germination. In general, two fundamentally different types of root systems can be found in the plant kingdom. The taproot system is characterized by an elongating primary root with developing lateral roots while in plants with a fibrous root system, the primary root is early on replaced by a plethora of shoot-borne roots. Recently our knowledge on the formation and patterning of the primary root meristem has improved seriously. From early embryogenesis onwards, a response maximum of the cell-fate instructive plant hormone auxin is formed that activates specific patterning genes and leads to the establishment of a root stem cell niche. During lateral and shoot-borne root formation, embryonic gene activities are employed again and as a consequence, these processes can be seen as a replay of embryonic pattern formation. Moreover, the onset of lateral and shoot-borne root formation from apparently fully differentiated cells is in sharp contrast with the embryogenic origin of the primary root and has been a point of controversy for many root biologists. In this review we will give an overview on the parallel mechanisms that might exist between embryonic and post-embryonic root development and will evaluate the potential existence of conserved molecular mechanisms between taproot versus fibrous root development.

Keywords: fibrous root system, dicots, lateral roots, monocots, primary root, taproot
Abbreviations: BFA, brefeldin A; **LR**, lateral root; **PR**, primary root; **PRM**, primary root meristem; **QC**, quiescent centre; **RAM**, root apical meristem; **SAM**, shoot apical meristem

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INTRODUCTION

The capacity to develop roots represented a fundamental evolutionary achievement enabling plants to migrate from aquatic to terrestrial habitats. In order to optimise their anchorage and uptake of water and nutrients, it is essential for terrestrial plants to develop an elaborate root system. Except for the primary root (PR), the entire root system of plants develops post-embryogenesis. This type of development is in contrast to animal development during which most of the body plan is established by the time embryogenesis is completed. Root systems vary widely both within and between species. One of the earliest root biologists, William Canon, made an initial distinction between root

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Invited Review

Fig. 1 Taproot versus fibrous root system. The taproot system of *Arabidopsis thaliana* (left) is characterised by one major primary root, from which lateral roots emerge shortly after germination. In contrast, the complex root system of maize (right) is mainly formed by shoot-borne roots. These shoot-borne roots are initiated from underground and aboveground nodes of the stem and are called crown and brace roots, respectively. The primary root system is only important during the very early stages of seedling development.

systems based on the PR that emerges from the seed (taproot system) and those that are based on shoot-borne roots (fibrous root system) (Canon 1949). Within the Angiosperms, dicotyledonous plants are characterised by a taproot system. Since most recent progress in both developmental and molecular events has been achieved in the dicot model plant *Arabidopsis thaliana*, we will focus on the latter as a representative member of plants with a taproot system. In accordance with the definition of a taproot system, the morphology of the *Arabidopsis* root system is characterised by one major PR, from which lateral roots emerge shortly after germination (**Fig. 1**). The fibrous root system is characteristic for most of the monocotyledonous plants. Due to the economical importance of the cereals maize and rice, molecular biologists over the last decade became increasingly attracted to study developmental aspects of monocot species. This has resulted in an increased insight into the fibrous root system anatomy and development. In contrast to *Arabidopsis*, in maize and rice the PR seems only to be important during the very early stages of seedling development whereas the functioning of the mature root system is guaranteed by multiple shoot-borne roots. These shoot-borne roots are initiated from underground and aboveground nodes of the stem and are called crown and brace roots, respectively. Some monocotyledonous plants, such as maize, form additionally embryonic roots. These seminal roots emerge a few days after germination from the scutellar node. All root types are able to develop lateral roots and form together the complex and intensively branched fibrous root system (Feix *et al*. 2002) (**Fig. 1**).

The existence of various types of taproot and fibrous root systems illustrates the diversity in root architecture that can be found across the plant kingdom. However at the anatomical level, roots of all plant species are remarkably similar. A central vascular bundle is surrounded, from inside to outside, by layers of four distinct cell types: pericycle, endodermis, cortex and epidermis (Dolan *et al*. 1993) (**Fig. 2**). The simple *Arabidopsis* root consists of one layer of each cell type, whereas the monocot maize contains 8 to 15 layers of cortical cells. The different cell types originate in the root meristem by the activity of a small population of stem cells. These stem cells surround a small group of mitotically less active cells, called the quiescent centre (QC) cells (Clowes 1956), that together act as an organizer to limit the stem cell status to its neighbouring cells (van den Berg *et al*. 1997). Depending on the species and the age of the individual root, the QC may vary from four cells in *Arabidopsis* (Dolan *et al*. 1993), to more than 1000 cells in maize (Jiang *et al*. 2003).

In this review, we describe the developmental biology of embryonic and post-embryonic root formation in *Arabidopsis* with emphasis on potential developmental and molecular pathways that might be shared between both processes. Furthermore, we discuss the level of conservation of the molecular mechanisms involved in root formation across monocotyledonous and dicotyledonous plants.

EMBRYONIC ROOT DEVELOPMENT

Building the primary root meristem

Both in monocots and in dicots, the primary root (PR) represents the first visible organ that can be recognized at the time of germination. The onset of PR development starts however much earlier during embryogenesis and in *Arabidopsis* these early developmental stages have been well-studied (Mayer *et al*. 1993; Scheres *et al*. 1994; Jürgens 2001). From fertilization on, the transition from zygote to embryo follows a conserved pattern of cell divisions starting with an asymmetric division that generates a small apical and a larger basal cell (**Fig. 3**). In most species, part of the descendants of both the basal cell and the apical cell will contribute to the embryonic root making the root a clonally hybrid plant organ. However, in species with a Caryophyllad type of embryogenesis, all root tissues derive from the apical cells (Barlow 2002). In *Arabidopsis* the apical cell forms a 32-cell globular structure by consecutive rounds of vertical and horizontal divisions, while the basal cell divides repeatedly horizontally yielding a single file of 7 to 9 suspensor cells (Jürgens 2001). At this stage, the uppermost suspensor cell is recruited by the apical proembryo and becomes specified as the founder cell of the PR meristem (PRM), or hypophysis. At heart stage, the hypophysis will divide asymmetrically giving rise to a large basal daughter cell and a lens-shaped apical daughter cell (**Fig. 3**). The apical cell will produce the four mitotically inactive cells of the quiescent centre (QC) that then induce their immediate adjacent cells to become the root meristem stem cells. Once the stem cells start to generate daughter cells, a fully functional root meristem is laid down.

Monocot embryogenesis differs severely from that of dicots and the architecture of monocotyledonous embryos is far more complex. Except for the first cell division that has been reported to be also asymmetric, the subsequent cell divisions follow a variable and unpredictable cell division pattern (even at the very early stages) during monocot embryogenesis (Zimmermann and Werr 2007). As a result, cell type specification in monocots is still poorly understood. The arbitrarily oriented divisions lead to a mass of cells that is defined as the pro-embryo in maize (Randolph 1936) and the early globular stage in rice (Itoh *et al*. 2005). In maize, the pro-embryo stage is followed by the transition stage where an internal, wedge-shaped meristematic region is formed in the anterior part of the embryo. At the coleoptilar stage, this region gives rise to the shoot apex, the surrounding coleoptilar ring, and the root apex. Similarly for rice, the formation of coleoptile, shoot apical meristem (SAM) and radicle primordium starts after the late globular stage. A major difference with dicots is that the PR of cereals is formed endogenously deep inside the embryo (Zimmermann and Werr 2005). Furthermore, the embryonic axis of monocotyledonous embryos is displaced laterally relative to

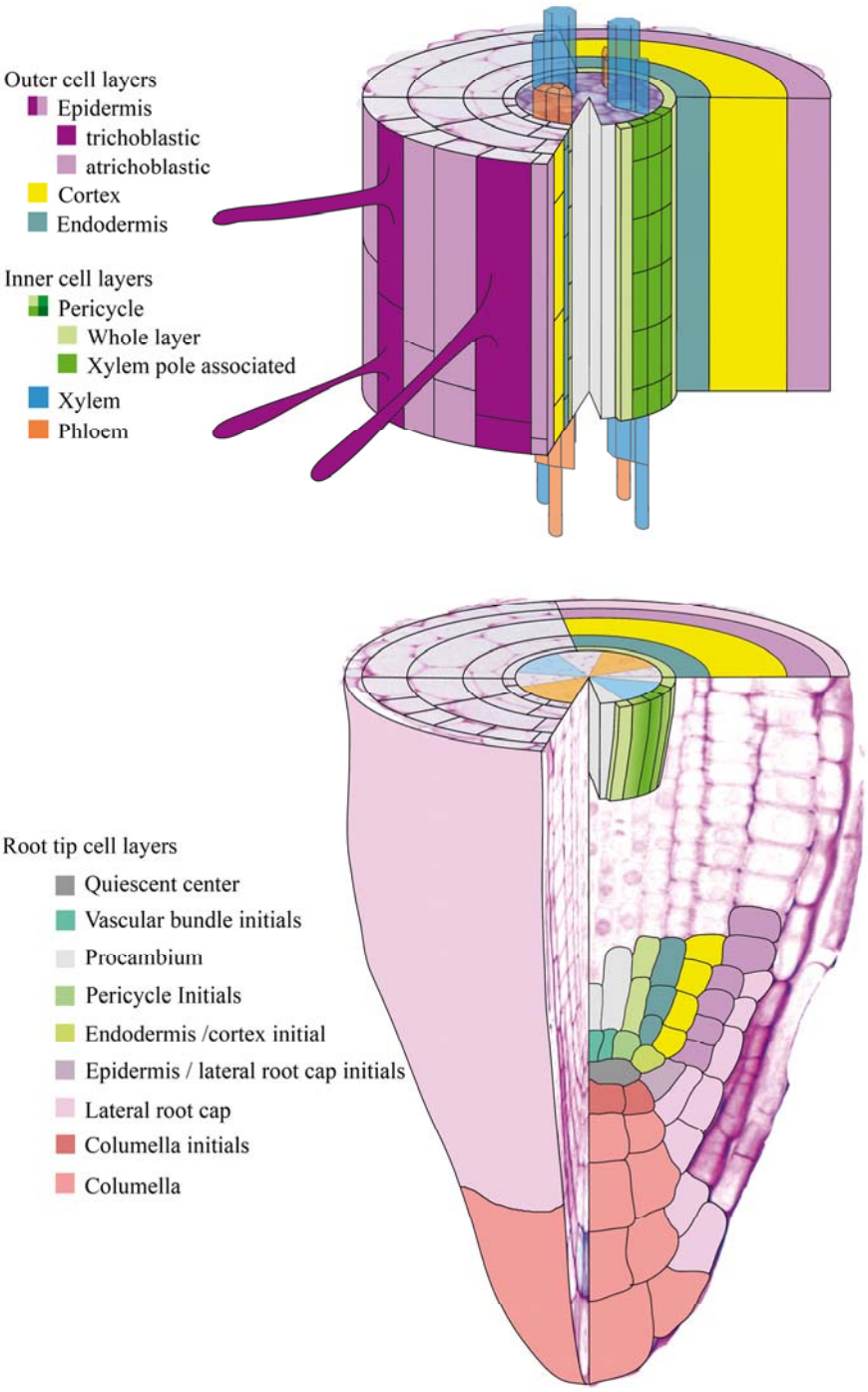

Fig. 2 Root anatomy of *Arabidopsis thaliana*. Upper panel: The central vascular bundle constituted from xylem and phloem, is surrounded by layers of four distinct cell types. From inside to outside: pericycle, endodermis, cortex and epidermis. Lower panel: The different root cell types originate in the root meristem by the activity of a small population of stem cells i.e. the root stem cell niche. The stem cells surround a small group of mitotically less active cells, called the QC cells.

the scutellum (which is considered to be the single cotyledon) in contrast to the apical-basal axis of dicots such as *Arabidopsis*.

Below we will give an overview on the molecular components involved in the formation of the embryonic root, first in *Arabidopsis* as representative species for the dicots, followed by a brief discussion of the more sparsely available data on monocot embryonic root development.

Arabidopsis root development: auxin as major player during hypophysis specification

The first anatomically recordable event in *Arabidopsis* root meristem formation is the establishment of the hypophysis. But how becomes the hypophysis specified? Recently, Friml *et al*. (2003) could demonstrate that during embryogenesis, efflux-dependent gradients of the phytohormone auxin fulfil a cell-fate instructive role. Using the auxin-responsive DR5 promoter activity, dynamic auxin accumulation patterns could be shown during embryogenesis, correlating with the localisation of auxin-transporting PIN proteins. The *Arabidopsis* genome contains 8 *PIN* genes of which 5 have been functionally characterised so far (PIN1, PIN2, PIN3, PIN4 and PIN7). These *PIN* genes encode transmembrane proteins mediating auxin efflux and present specific and overlapping expression domains (Paponov *et al*. 2005). Two of them, *PIN7* and *PIN1*, are expressed during early embryo development. PIN7 is localized to the apical plasma membranes of the suspensor cells suggesting that PIN7 facilitates auxin transport from the maternal tissue to the apical pro-embryo (Friml *et al*. 2003). In the pro-embryo itself auxin is equally distributed by PIN1. Inhibition of the apical auxin accumulation using auxin transport inhibitors or mutants in *PIN7*, disturbs the apical-basal axis formation and subsequently, the conserved cell division program (Friml *et al*. 2003). At the globular stage, the direction of PIN1-mediated auxin transport in the pro-embryo flips and the combined action of PIN1, PIN7 and a third PIN-family member, PIN4 leads to an auxin accumulation in the uppermost suspensor cell inducing hypophysis specification (Friml *et al*. 2003). Due to functional redundancy among PIN proteins, single, double and triple mutants show

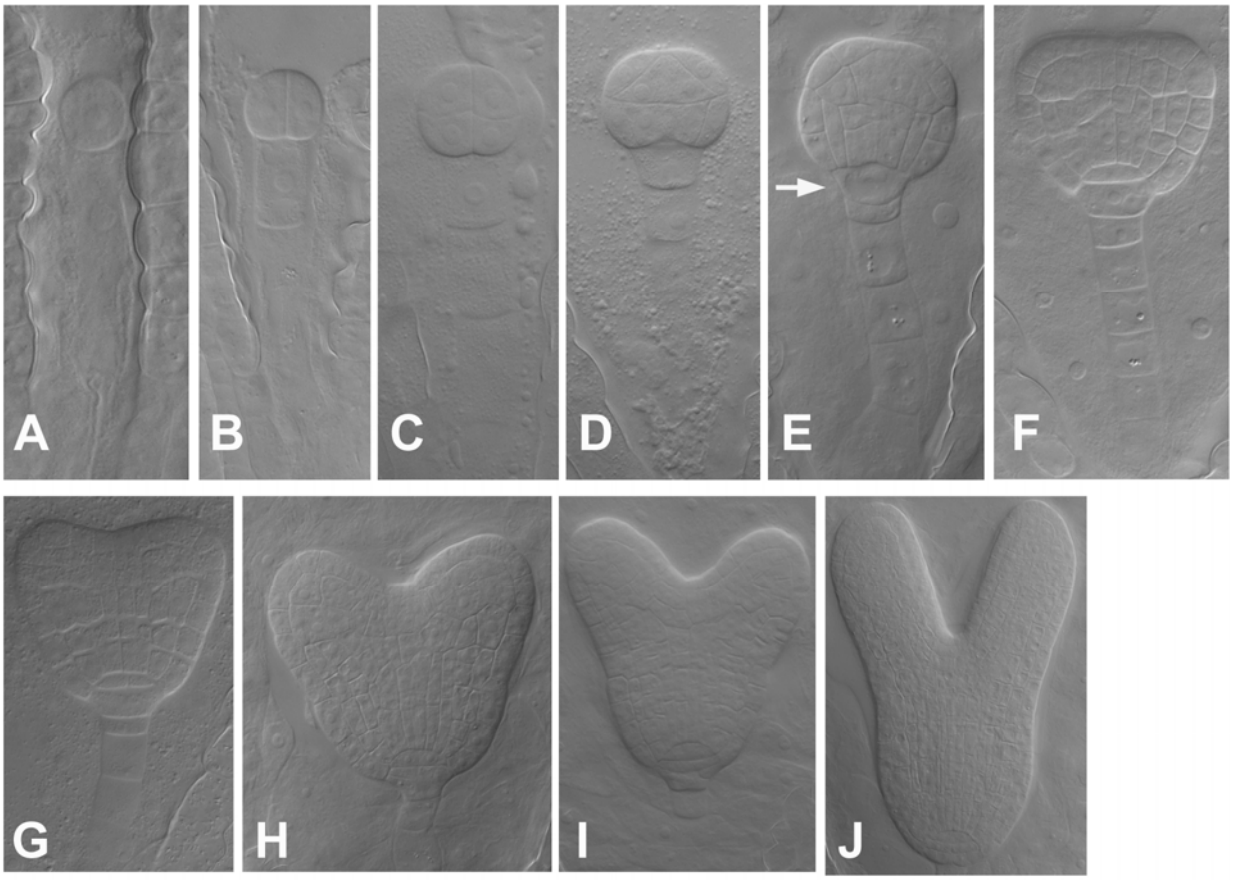

Fig. 3 Embryogenesis of *Arabidopsis thaliana*. After fertilization the zygote will divide asymmetrically yielding a small apical and a larger basal cell. This stage is described as the one-cell stage (**A**). While the basal cell will divide horizontally, the apical cell divides vertically resulting in the two-cell stage (**B**). Consecutive rounds of vertical and horizontal divisions of the apical cells result in the octant stage (**C**), dermatogen stage (**D**) and ultimately in the globular stage (**E**). The basal cells divide repeatedly horizontally yielding a single file of 7 to 9 suspensor cells. Subsequently the hypophysis (indicated with white arrow) divides asymmetrically giving rise to a large basal daughter cell and a lens-shaped apical daughter cell. This stage is referred to as the triangular stage (**F**). The apical cell will produce the four mitotically inactive cells of the QC (**G**). The embryos develop into a heart stage (**H**) and later into the torpedo stage (**I**, early torpedo; **J**, torpedo). During this transition the root stem cells start to generate daughter cells and a fully functional root meristem is laid down.

no (Blilou *et al*. 2005) or rather subtle defects (Friml *et al*. 2003; Vieten *et al*. 2005). However, quadruple mutants *pin1 pin2pin3pin7* and *pin2pin3pin4pin7* are embryo-lethal and show aberrant cell divisions from the first cell divisions on, leading to malformed globular embryos. These early dramatic defects clearly highlight the importance of efflux-dependent auxin gradients for embryonic development (Friml *et al*. 2003; Blilou *et al*. 2005; Vieten *et al*. 2005). In order to react to developmental (such as PIN1 polarity switch during embryogenesis) or external cues (such as gravitropic response, see below), the polarity of PIN proteins can be rapidly modulated. The PIN proteins are continuously cycling between the plasma membranes and endosomal compartments, a process that is enabled by the *GNOM* gene. *GNOM* encodes a membrane-associated GDP/GTP exchange factor for small G proteins of the ARF class (ARF-GEF) and regulates vesicle transport that is sensitive to the drug brefeldin A (BFA) (Steinmann *et al*. 1999; Geldner *et al*. 2003). In strong *gnom* mutants the coordinated localization of PIN1 is perturbed and as a result these embryos display similar phenotypes as quadruple *pin* mutant embryos or embryos treated with auxin transport inhibitors, showing aberrant cell divisions throughout embryogenesis (Mayer *et al*. 1993). As a consequence *gnom* mutants fail to specify the hypophysis and result in seedlings without a functional root apical meristem (RAM) (Mayer *et al*. 1993). Interestingly the earliest abnormalities were observed during the one-cell stage, in which the plane of division was nearly symmetrical, suggesting that the *GNOM* gene is required before the asymmetric division of the zygote (Mayer *et al*. 1993). Although more PIN proteins have been shown to cycle rapidly and internalize upon BFA treatment, GNOM does not seem to mediate all PIN protein trafficking to the same extent (Geldner *et al*. 2003; Jaillais *et al*. 2006).

The significance of auxin accumulation in the uppermost suspensor cell coinciding with hypophysis specification could be illustrated by the PP2A phosphatase mutants and by the ectopic expression of the PINOID protein kinase (PID) during early embryogenesis. Both enzymes control antagonistically the PIN apical-basal targeting (Friml *et al*. 2004; Michniewicz *et al*. 2007). Conditions in which PID kinase activities are relatively high result in predominantly phosphorylated PIN proteins, causing their targeting to the apical side of cells. In the converse situation, when PID activities are lower than those of PP2A phosphatase, PIN proteins are dephosphorylated and targeted preferentially to the basal side of the cell. In *PID*-overexpressing embryos and in *pp2aa* double mutant embryos, the characteristic turnover in auxin transport direction during globular stage does not occur and consequently an auxin maximum in the uppermost suspensor cell is not installed. This results in a misspecification of the hypophysis and consequently in rootless seedlings (Friml *et al*. 2004; Michniewicz *et al*. 2007).

In addition to a correctly organized auxin transport mechanism, the importance of auxin in hypophysis specification has also been demonstrated at the level of auxin response. Mutations in two genes, *MONOPTEROS* (*MP*) and *BODENLOS* (*BDL*) affect the formation of the RAM. Instead of the asymmetric horizontal division of the hypophysis, *mp* and *bdl* embryos show a vertical division, which leads to seedlings lacking an embryonic root (Hamann *et al*. 2002). The *MP* gene encodes ARF5, a transcription factor

of the ARF (auxin response factor) family that activates auxin-responsive target genes (Hardtke and Berleth 1998). *BDL* encodes IAA12, a member of the Aux/IAA family of which all members act as negative regulators of the ARFs, by heterodimerization at low auxin concentrations. High auxin levels directly activate the SCF^{TIR1} E3 ubiquitin ligase complex that promotes proteasome-mediated degradation of Aux/IAA members allowing the ARFs to regulate their auxin target genes (Gray *et al*. 2001). BDL and MP were shown to interact *in planta* and are (at least partially) responsible for the auxin response from early embryogenesis on (Weijers *et al*. 2006). Stabilization of the BDL homologue IAA13 prevents also MP-dependent embryonic root formation and as the expression pattern of *IAA13* overlaps with that of *BDL*, it is assumable that both proteins need to be degraded for activation of MP target genes (Weijers *et al*. 2005). Interestingly *MP* and *BDL* (and *IAA13*) are expressed only in the pro-embryo and lack expression in the hypophysis. Furthermore they are not able to move from the pro-embryo to the hypophysis, suggesting they might regulate hypophysis specification in a non-cell-autonomous way (Weijers *et al*. 2006). Currently, intensive research focuses on trying to identify the MP/BDL downstream transcription factors, which transmit the cell-fate-instructive signal to the hypophysis (D. Weijers, pers. comm.).

More mutants for components of the auxin-mediated signalling show defects in the hypophyseal cell lineage. These include mutations in the *CUL1/AXR6* gene (Hobbie *et al*. 2000; Hellmann *et al*. 2003) and the *CUL3A* and *CUL3B* genes (Thomann *et al*. 2005), which all encode CULLIN subunits of the auxin-regulated SCF^{TIR1} ubiquitin E3 ligase complex. Consistently, similar defects could be observed in *tir1afb2afb3* embryos mutated in auxin-signalling F-box proteins (Dharmasiri *et al*. 2005). Interestingly, other multimeric complexes involved in protein degradation are important for proper hypophysis formation. The *HOBBIT* (*HBT*) gene (Willemsen *et al*. 1998) encodes a homolog of a CDC27 anaphase-promoting complex (APC)/cyclosome subunit. The mutational defects become apparent around octant stage in which the uppermost suspensor cell divides vertically rather than horizontally. Later on, atypical divisions occur in the hypophyseal cell region. Postembryonically, *hbt* seedlings are characterized by a small embryonic root with no mitotic activity and with the absence of a differentiated lateral root cap. A detailed analysis of the *hbt* mutant using the expression of several pattern markers led Blilou *et al*. (2002) to conclude that it is more likely that HBT is required for progression of cell differentiation rather than for pattern formation. The defects in the *hbt* root meristem correlate with an accumulation of the Aux/IAA protein IAA17/AXR3 and a reduction of IAA3/SHY2, suggesting that an altered auxin response could be the reason for the developmental defects (Blilou *et al*. 2002; Serralbo *et al*. 2006). However using a mosaic analysis, in which inducible HOBBIT loss-of-function clones were generated by a Cre/lox-mediated recombination, Serralbo *et al*. (2006) could demonstrate that the changes in auxin response occur well after the cell division and cell expansion defects. Since *HBT* encodes a subunit of the APC complex that regulates G2/M transition during the cell cycle, it clearly shows the involvement of the cell cycle in embryonic patterning. This relation could also be demonstrated by the characterization of the *tilted1* mutant (Jenik *et al*. 2005). *TILTED1* encodes the catalytic subunit of DNA-polymerase ε of *Arabidopsis thaliana*, necessary for DNA synthesis during the S phase of the cell cycle. Mutating this gene leads to a lengthening of the cell cycle throughout embryo development and alters cell type patterning of the hypophyseal lineage in the root.

Specification of the root stem cell niche

In the previous paragraphs we emphasized the importance of intact auxin responses and correct auxin distribution patterns to generate the PR axis during early embryogenesis. The obvious next question is: how are the auxin responses translated into cell patterns and QC formation that are essential to establish a fully functional RAM? During the last years, our insight into the mechanisms of pattern formation has increased seriously by the study of additional *Arabidopsis* mutants. The auxin accumulation seems to be interpreted by the hypophyseal derivatives through auxin-induced expression of the *PLETHORA* (*PLT*) genes. These genes encode putative AP2 (APETALA2)-type transcription factors and form a small subfamily within the large AP2/EREBP (ethylene-responsive element binding proteins) family. The *PLT1* and *PLT2* genes are expressed in the basal half of the proembryo as early as in the octant stage and their expression depends on the auxin response transcription factors MP/ARF5 and its homologue NPH4/ ARF7 (Aida *et al*. 2004). In the absence of both PLT1 and PLT2 function, the hypophyseal derivatives divide abnormally and fail to establish the QC (Aida *et al*. 2004), indicating that the *PLT* genes are essential for QC specification and stem cell activity. Very recent research results have revealed that PLT1 and PLT2 act redundantly with two other PLT homologues, PLT3 and BBM (BABY BOOM) (Galinha *et al*. 2007). When knocking-out the expression of all four genes of the PLT clade, quadruple mutants with no root and hypocotyl were obtained. These defects were initiated during early embryogenesis and mimic the defects observed in mutants impaired in auxin signalling or perception (see above). Interestingly the *PLT* genes also regulate *PIN* gene expression in the RAM thereby stabilizing and fine-tuning the position of the stem-cell-associated auxin maximum (Blilou *et al*. 2005; Galinha *et al*. 2007). However, besides auxin, additional positional information is needed to prepattern specific cell layers. This information is (at least partially) delivered by the auxin-independent expression of *SHORTROOT* (*SHR*) and *SCARECROW* (*SCR*) that encode members of the GRAS family of putative transcription factors. Both genes are essential for radial patterning from heart stage on, where SHR specifies endodermal cell fate and SCR controls the periclinal division of the cortex-endodermal initial cell (Scheres *et al*. 1995; Di Laurenzio *et al*. 1996; Helariutta *et al*. 2000; Wysocka-Diller *et al*. 2000). *SHR* is expressed exclusively in the provascular cells (vascular tissue in seedlings), but the protein moves to the surrounding endodermal cell layer including the QC where it promotes *SCR* transcription (Nakajima *et al*. 2001; Levesque *et al*. 2006). Very recently, it has been demonstrated that SCR and SHR interact in yeast and that the SHR/SCR complex acts as positive feedback mechanism for *SCR* transcription (Cui *et al*. 2007). Furthermore, SCR regulates the subcellular localisation and movement of SHR. SCR knock-down seedlings with still enough SCR to stimulate the SCR/SHR transcriptional network in the endodermis but not enough SCR to sequester SHR in the nucleus, have super-numerary endodermal cell layers and prove that SCR prevents SHR from moving to cell layers outside the endodermis by directing SHR to the nucleus. Although *scr* and *shr* mutants show no defects in apical-basal patterning during embryogenesis, SCR is cell-autonomously required for QC identity and stem cell specification during post-embryonic root development (Sabatini *et al*. 2003; Heidstra *et al*. 2004). Interestingly, *SCR* expression in the stem cells of *scr* mutants is insufficient for their maintenance, suggesting that SCR activity in the QC is required to maintain the surrounding cells in a stem cell state (Sabatini *et al*. 2003). Since expression of *SCR* in the QC of *shr* mutants is also not sufficient to restore QC and stem cell identity (Sabatini *et al*. 2003), it is plausible that SHR also activates the still unknown short-range signal transmitted by the QC to the surrounding stem cells (see below). Through the generation of double and triple mutants, Aida *et al*. (2004) were able to show that the *PLT* genes act in parallel with the SCR/SHR pathway in such a way that the transcriptional overlap of the 3 putative transcription factors defines the QC cells. Consequently, ectopic embryonic expression of the *PLT* genes specifies new QC and stem cells in any position where *SCR* and *SHR* are expressed, resulting in extreme

situations such as the transformation of cotyledons and/or SAM to ectopic roots with fully active root meristems (Aida *et al*. 2004; Galinha *et al*. 2007).

In conclusion, the above mentioned recent findings make it possible for the first time to propose a model that describes a molecular network that controls the formation of the embryonic root. In this model, a PIN-mediated auxin maximum is instructive for the expression of the *PLT* genes that define the stem cell region in concert with SHR and SCR and in turn, control the root-specific *PIN* expression to stabilize the auxin maximum (Blilou *et al*. 2005).

Embryonic root formation in monocotyledonous plants

Our rapidly increasing insight into root development based on genetic studies is mainly restricted to *Arabidopsis* and thus dicot roots. Root formation in monocotyledonous embryos is however far less understood. Additionally, in some monocots such as maize, embryogenesis gives rise to additional roots besides the PR, generally referred to as seminal roots. The primordia of these roots are established in variable numbers at the scutellar node during the late phases of embryogenesis. In spite of the strong differences during embryogenesis and the morphological differences of *Arabidopsis* and cereal roots, several indications imply a conserved genetic mechanism for the establishment of at least the root radial organisation in monocots. Genes closely related to *AtSCR* have been identified in maize and rice (Lim *et al*. 2000; Kamiya *et al*. 2003; Lim *et al*. 2005; Cui *et al*. 2007) showing a similarly specific expression pattern in the endodermal cell layer of the PR, in the seminal roots of maize, in the post-embryonic crown and lateral roots as well as during embryogenesis (Zimmermann and Werr 2005). Moreover, *ZmSCR* expressed under the control of the native *AtSCR* promoter can rescue the patterning defects observed in *Arabidopsis scr* mutants (Lim *et al*. 2005). Recently, Cui *et al*. (2007) identified *OsSHR1* as the functional homologue of *AtSHR* in rice and analogously to *OsSCR1* and *ZmSCR*, it is expressed in the same tissues as their counterparts in *Arabidopsis*. Furthermore, in yeast *OsSHR1* interacts with *OsSCR1* as well as with *AtSCR*. Taken together, these recent findings imply an evolutionarily conserved SHR/SCR-regulated mechanism and might provide a plausible explanation for the occurrence of only one single endodermis layer in all Angiosperms (Cui *et al*. 2007). Besides the SHR/SCR signalling, the auxin signalling transduction mechanisms might also be well-conserved between monocot and dicot plants. A comparative study of the primary structures of *Arabidopsis* and rice *ARF* genes revealed that rice contains one or two closely related orthologous gene(s) corresponding to each respective *Arabidopsis ARF*, including *MP* (Sato *et al*. 2001). This suggests that the functions of the corresponding ARF proteins in *Arabidopsis* and rice may be similar to each other. Additionally using the *radicleless1* (*ral1*) mutant, Scarpella *et al*. (2003) provided genetic evidence that auxin sensitivity is associated with embryonic root development in rice. In maize, Scanlon *et al*. (2002) could show that a reduction in polar auxin transport in the *semaphore1* (*sem1*) mutants affects embryo and lateral root development.

In contrast to the limited number of known auxin-related mutants, large numbers of mutants with specific defects in embryogenesis have been isolated and analysed in monocots (Clark and Sheridan 1991; Hong *et al*. 1995; Consonni *et al*. 2003; Scarpella *et al*. 2003; Kinae *et al*. 2005). These mutants are commonly classified as *emb* (embryo-specific), for mutants characterised by an impaired or arrested embryo development and a normal endosperm, and as *dek* (defective kernel) when impaired in both endosperm and embryo and, less frequently, only in endosperm development. A few mutants showing abnormal seedling and plant morphology, in which defects can be traced back to earlier events occurring during embryogenesis, have also been identified (Dolfini *et al*. 1999; Landoni *et al*. 2000) and are referred to as *des* (defective seedling). However to our knowledge, genes corresponding to these mutants have not been identified so far. In the future, identification of the corresponding genes will make it possible to compare the formation of the embryonic roots of dicots and monocots in more detail at the molecular level.

POST-EMBRYONIC ROOT GROWTH

Physiological control of root meristem maintenance

Post-embryonic root growth is supported by the root apical meristem (RAM). In the meristem, stem cells continuously produce daughter cells by asymmetric cell divisions, and once the daughter cells leave the meristematic zone they support root growth by cell elongation. The organizing auxin maximum at the root stem cell niche installed during embryogenesis, is maintained throughout post-embryonic development and was shown to be critical for post-embryonic root meristem maintenance in *Arabidopsis* (Sabatini *et al*. 1999). Sabatini *et al*. (1999) showed that the stem cell niche in the *Arabidopsis* root nicely coincides with an auxin response maximum and that the latter is required for correct specification of root cell fates. A reduction or displacement of the auxin gradient causes dramatic changes in patterning of the root tip (Sabatini *et al*. 1999; Benjamins *et al*. 2001; Friml *et al*. 2002), whereas increasing the auxin maximum by treatment with polar auxin transport inhibitors induces ectopic QC formation (Sabatini *et al*. 1999). Immunolocalisation of auxin in maize roots revealed that the root cap and QC contained relatively higher levels of auxin (IAA) than the immediately surrounding cells arguing for the existence of a similar mechanism in monocot roots (Kerk and Feldman 1995).

During post-embryonic plant growth, auxin is mainly produced by the aerial parts of the plant, especially in young developing leaves, but also in the RAM and in young lateral roots (Ljung *et al*. 2001, 2005). However, the synthesis capacity in the root tip, although significant, is not high enough to maintain the organizing auxin gradient in the root tip especially during early seedling development (Ljung *et al*. 2005). Therefore IAA derived from the shoot is transported to the root tip by means of a complex interacting network of influx and efflux systems. Transmembrane proteins of the AUX1/LAX family were shown to be part of the influx system (Bennett *et al*. 1996; Swarup *et al*. 2001; Yang *et al*. 2006), whereas the PIN proteins (see above) were shown to mediate auxin efflux (Paponov *et al*. 2005). In *Arabidopsis*, at least five PIN proteins are expressed in specific but partially overlapping regions of the RAM (Paponov *et al*. 2005) and by means of their asymmetric subcellular localisation patterns they are able to give directionality to auxin transport (Wisniewska *et al*. 2006) leading to the establishment of auxin maxima. Also in other species *PIN* genes were identified, such as *ZmPIN1a* and *ZmPIN1b* in maize (Carraro *et al*. 2006) and *OsPIN1* in rice (Xu *et al*. 2005). Over the last years, a group of ABC transporters belonging to the multidrug resistant (MDR)-like family also known as the P-glycoproteins (PGPs), emerged as new players in the cellular efflux and influx of auxin (Noh *et al*. 2001; Blakeslee *et al*. 2005; Geisler *et al*. 2005; Terasaka *et al*. 2005). Loss-of-function mutations in several *PGP* genes cause diverse developmental defects that are related to altered auxin signalling and/or distribution, and some show aberrant polar auxin transport and altered auxin uptake or efflux from leaf protoplasts (Noh *et al*. 2001; Geisler *et al*. 2005; Santelia *et al*. 2005; Terasaka *et al*. 2005; Lewis *et al*. 2007). However these developmental aberrations are often distinct from those found in *pin* mutants or those induced by the chemical inhibition of auxin transport, suggesting that PGPs potentially have additional, unknown functions that might be unrelated to auxin transport. On the other hand in heterologous systems such as yeast or mammalian HeLa cells, some PGPs are capable of transporting different

auxins across the plasma membrane out of the cell (PGP1/PGP19) or into it (PGP4) (Geisler *et al*. 2005; Santelia et al 2005; Terasaka *et al*. 2005), and these data are at least as convincing as those obtained for the PINs. Mutations in *PGP* genes have been identified in maize (*brachytic2/zmpgp1*), sorghum (*dwarf3/sbpgp1*) and rice (Multani *et al*. 2003; Geisler and Murphy 2006), demonstrating a conserved role for the PGP proteins. Until now the relation between the PGPs and PINs is still unclear. Although it was shown that PIN1 action on plant development does not strictly require function of PGP1 and PGP19 (Petrasek *et al*. 2006), it is likely that the different auxin transport systems are coordinated and functionally interact. Recently it was demonstrated that PINs and PGPs can interact with each other and that PGP proteins act synergistically with PIN proteins in transporting auxin (Blakeslee *et al*. 2007). One possible explanation is that PGP proteins increase the stability of the PIN proteins on the plasma membranes (Noh *et al*. 2003; Petrasek *et al*. 2006; Blakeslee *et al*. 2007). Stability and composition of the plasma membrane is indeed important for correct polar auxin transport as could be demonstrated by a mutation in STEROL METHYL TRANSFERASE1 (SMT). The corresponding *orc* mutant is characterised by defective pattern formation from embryogenesis on, mostly by an affected polar localization of PIN proteins (Willemsen *et al*. 2003). *SMT1* encodes an enzyme that is involved in the production of membrane sterols, highlighting that a balanced sterol composition of the plasma membrane is a major requirement in polar targeting of PIN proteins.

In the root, the shoot-derived auxin is transported through the vascular and provascular cells towards the tip by the combined action of PIN1, PIN3 and PIN7 (**Fig. 4**). Then, PIN4 delivers the auxin to the central columella cells where the combined action of PIN3, PIN4 and PIN7 maintain the position of the auxin maximum and redistribute auxin laterally. PIN2 together with the influx carrier AUX1 (see above) then stimulates acropetal transport through the epidermis towards the elongation zone where auxin can be reloaded into the vascular system facilitated by PIN1, PIN2, PIN3 and PIN7 (**Fig. 4**). This auxin reflux loop is not only essential to maintain the auxin maximum at the stem cell niche, but recently Blilou *et al*. (2005) could show that the root meristem size of all double and triple mutants containing *pin2* was significantly reduced. This suggests that basipetal auxin transport to the meristematic cells plays a critical role in the regulation of meristem length and thus in root growth, and that control of cell division is a major factor in this process (Blilou *et al*. 2005). Moreover Blilou *et al*. (2005) could show that the PIN proteins also regulate cell expansion and the size of the root elongation zone. These observations match perfectly with very recent data obtained by Galinha *et al*. (2007) and Grieneisen *et al*. (2007). Grieneisen *et al*. (2007) developed a model for polar auxin transport during root growth and hypothesised that the well-known auxin maxima are in fact associated with auxin gradients (such as the auxin maximum at the stem cell niche of the PR reflects the maximum of an auxin gradient throughout the PR meristem). Intriguingly Galinha *et al*. (2007) showed that during post-embryonic root development the *PLT* genes and homologues (see above) display gradients in both promoter activity and protein concentration making them good candidates to represent a readout of the root auxin gradient. Interestingly, the PLT gradients are translated into distinct cellular responses. The highest PLT activity coincides with the auxin maximum in the stem cell area and promotes stem cell identity and maintenance. The gradients fade out throughout the meristem where these lower levels promote the mitotic activity of the meristematic cells. Indeed when overexpressing *PLT2* in the root, the meristem size highly increased while a reduction of the PLT gradient reduced the meristem size (Galinha *et al*. 2007). Ultimately the concentration gradient ends in the elongation zone where very low PLT protein levels lead to cell differentiation. Based on their results, Galinha *et al*. (2007) state that the PLT proteins act through auxin as dose-dependent master regulators of root development in *Arabidopsis*.

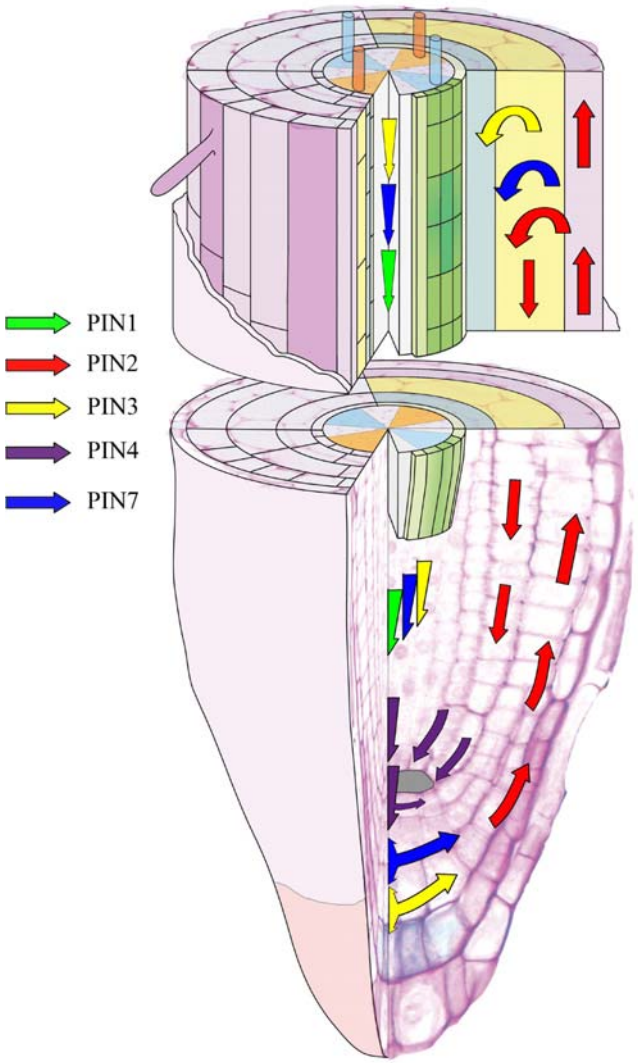

Fig. 4 Schematic representation of polar auxin transport in the RAM. In the root, the shoot-derived auxin is transported through the vascular and provascular cells towards the root tip by the combined action of PIN1, PIN3 and PIN7. Then, PIN4 delivers the auxin to the central columella cells where the combined action of PIN3, PIN4 and PIN7 maintain the position of the auxin maximum and redistribute auxin laterally. PIN2 then stimulates acropetal transport through the epidermis towards the elongation zone where auxin can be reloaded into the vascular system facilitated by PIN1, PIN2, PIN3 and PIN7.

Molecular control of root meristem maintenance

Laser ablation studies have demonstrated that the QC cells generate short-range signals that prevent differentiation of their neighbouring stem cells (van den Berg *et al*. 1997). Moreover it has been demonstrated that during regeneration of the root tip after surgical excision (Feldman 1976; Rost and Jones 1988) or laser ablation of the QC (Xu *et al*. 2006), the reformation of a QC precedes and is essential for the organisation of the new RAM. Until now the mysterious signals transmitted by the QC remain unknown. However, the ongoing stem cell research of the SAM during the last decade lifted a tip of the veil. In *Arabidopsis* shoot meristems, control of the size and positioning of the stem cell niche is controlled by a two-way signalling between the organizing centre and overlying stem cells. Stem cells produce a small, secreted protein CLAVATA3 (CLV3) that activates the CLV1/CLV2 receptor complex in turn controlling the size of the organizing centre by transcriptional inhibition of WUSCHEL (WUS) (Laux 2003). Interestingly, root

meristem-specific overexpression of *CLE19*, which encodes a *CLV3* homolog, reduces the size of the root meristem and ultimately the root meristem differentiates (Casamitjana-Martinez *et al*. 2003; Fiers *et al*. 2004). These root defects are not due to a misspecification of the QC or a loss of initials, but to a defect in meristem maintenance (Casamitjana-Martinez *et al*. 2003). The same results could also be obtained by ectopic expression of *CLV3* and *CLE40* (Hobe *et al*. 2003) and strongly argue for a role of a CLV-like pathway in root meristem maintenance. By *in vitro* application of CLV3, CLE19 and CLE40 peptides and the use of *clv* mutants, Fiers *et al*. (2005) proposed a model in which the CLE peptides interact with or saturate a CLV2 receptor complex in roots, leading to consumption of the root meristem.

More evidence for an equivalent regulation of stem cell identity in the RAM and SAM is provided by the functional analysis of the *WOX* genes (Haecker *et al*. 2004). *WOX* or *WUS*-related homeobox genes show a very distinct expression pattern during embryogenesis. One member, *WOX5*, is auxin-inducible (Gonzali *et al*. 2005) and is expressed very early in the hypophyseal cell (Sarkar *et al*. 2007). *WOX5* expression marks the identity of the QC from embryogenesis on (Haecker *et al*. 2004; Sarkar *et al*. 2007) throughout post-embryogenic development (Blilou *et al*. 2005; Sarkar *et al*. 2007). Promoter swapping experiments have demonstrated that the expression of *WOX5* under control of the *WUS* promoter can rescue the stem cell defects in the *wus* mutant and, *vice versa*, QC specific expression of *WUS* can compensate for the loss of *WOX5* function (Sarkar *et al*. 2007). In rice, Kamiya *et al*. (2003) identified a QC-specific homeobox gene (*QHB*), which is closely related to *AtWOX5* (Haecker *et al*. 2004). Similarly to *AtWOX5*, in rice *QHB* is specifically expressed in the central cells of the QC of the root and could first be detected in the basal region of the embryo prior to the morphological differentiation of the radicle (Kamiya *et al*. 2003). Expression of *AtWOX5* is predominantly regulated by the SHR/SCR pathway, identifying WOX5 as one of the downstream effectors of the SHR/SCR signalling pathway in stem cell maintenance. The function of this QC marker was unknown for a long time, but very recently Sarkar *et al*. (2007) could demonstrate that *AtWOX5* inhibits the differentiation of the root stem cells. Loss of WOX5 function causes terminal differentiation of the columella stem cells, while gain of function blocks differentiation as could be demonstrated by an indefinite number of columella stem cells. Furthermore, this block of differentiation is independent from any further QC signalling, and as such WOX5 might provide us with a tool to identify the unknown short-range factor(s) transmitted from the QC to the stem cells (van den Berg *et al*. 1997). Most likely WOX5 activates downstream signals in the QC, which then move to the stem cell population to inhibit their differentiation. However, since cellular localisation data of the WOX5 protein is not available until now (Sarkar *et al*. 2007), the WOX5 protein could also move to the stem cells and could be the mysterious signal itself.

POST-EMBRYONIC ROOT FORMATION

As the PR grows, lateral roots (LRs) will emerge from the PR shortly after germination. The process of LR formation has been well studied in many plants, including *Arabidopsis*, and multiple studies have shown that auxin is a key regulator of LR formation and development (Blakely *et al*. 1982; Laskowski *et al*. 1995; Casimiro *et al*. 2001; Benkova *et al*. 2003). It is well established that exogenous application as well as overproduction of auxin leads to supernumerary LRs (Boerjan *et al*. 1995; Celenza *et al*. 1995; King *et al*. 1995), while auxin transport inhibitors block LR initation (Casimiro *et al*. 2001).

Lateral roots originate from the pericycle cells adjacent to the xylem pole in most dicotyledonous plants or to the phloem pole in monocotyledonous plants and some dicotyledonous species (Casero *et al*. 1995; Lloret and Casero 2002; Feix *et al*. 2002). LRs emerge in an acropetal order, with longer LRs at the most basal region and progressively shorter LRs toward the PR tip, and are spaced along the main axis in a regular left-right alternating pattern. Interfering with the root vascular initials can have dramatic consequences for LR formation. In the *Arabidopsis lonesome highway* (*lhw*) mutant, PR and LRs produce only one instead of 2 files of xylem, phloem and LR producing pericycle cells. This results in a root system with LRs from only one side of the PR (Ohashi-Ito and Bergmann 2007). *LHW* encodes a member of a novel, plant-specific family of putative transcription factors in *Arabidopsis* but interestingly is also represented in the monocot rice. Additionally *lhw* is not able to maintain the RAM, fails to express *SCR* in the QC and thus illustrates the close relationship between PR and LR development. In the next paragraphs we will describe post-embryonic root development and will highlight the parallel mechanisms that might exist between embryonic and post-embryonic root development.

Building a lateral root meristem

Besides the importance of auxin, remarkable morphological similarities can be seen when comparing LR (post-embryonic) with PR (embryonic) formation. Similar to embryogenesis, the first recognisable hallmark of LR formation is the appearance of an asymmetric cell division event. Two pericycle founder cells within the same cell file undergo almost simultaneously an asymmetric transverse/anticlinal division yielding two short cells flanked by two longer ones (Malamy and Benfey 1997; Dubrovsky *et al*. 2000; Casimiro *et al*. 2001; Casimiro *et al*. 2003). This division pattern can be viewed microscopically (**Fig. 5**), is referred to as 'initiation' and has been reported from several plant species (Casero *et al*. 1995). Based on DR5-GUS marker studies in *Arabidopsis* that are indicative for auxin distribution patterns at the tissue level, it was proposed that auxin might accumulate in the pericycle founder cells prior to the formative asymmetric divisions (Benkova *et al*. 2003). Still in *Arabidopsis*, once initiation has occurred, the founder cells begin a well-conserved program of cell divisions orchestrated by PIN-dependent auxin transport to form an LR primordium (Malamy and Benfey 1997; Casimiro *et al*. 2001; Benkova *et al*. 2003). Although *PIN1*, *PIN3*, *PIN4*, *PIN6*, and *PIN7* are all expressed during the earliest developmental stages, only a detailed localisation study of PIN1 is available until now (Benkova *et al*. 2003). While after the first round of asymmetric division the short daughter cells continue to divide anticlinally, thereby creating a group of maximum 10 short cells with similar length, PIN1 localises exclusively to the transverse (anticlinal) sides of the daughter cells creating an auxin maximum in the central cells. These central daughter cells expand radially and divide periclinally, giving rise to a stage II primordium composed of an inner and an outer cell layers. During stage III and IV, both the outer and inner layers divide further periclinally and anticlinally giving a four-cell layered primordium (Malamy and Benfey 1997) (**Fig. 5**). Cell division activity is high in the centre of the developing primordia while some peripheral cells of the outer cell layer do not divide. This gradient in cell division activity with a maximum in the centre and decreasing towards the periphery is interpreted to be essential in creating and maintaining the shape of the primordium. From stage II on, PIN1 polarity is redirected from the transverse to the lateral sides of the central cells of the primordium, providing auxin from the PR vasculature to the primordium tip where it accumulates in the central cells of the outer layers (Benkova *et al*. 2003). Comparable to early embryogenesis (prior to triangular/heart stage), the LR primordium consists of a homogenous group of dividing cells without visible morphological differentiation. At stage V two central cells, one in each of the two outer layers divide anticlinally to form four small cubical cells. Simultaneously the cells of the inner layers enlarge radially and divide periclinally pushing the overlying layers through the parent root. During

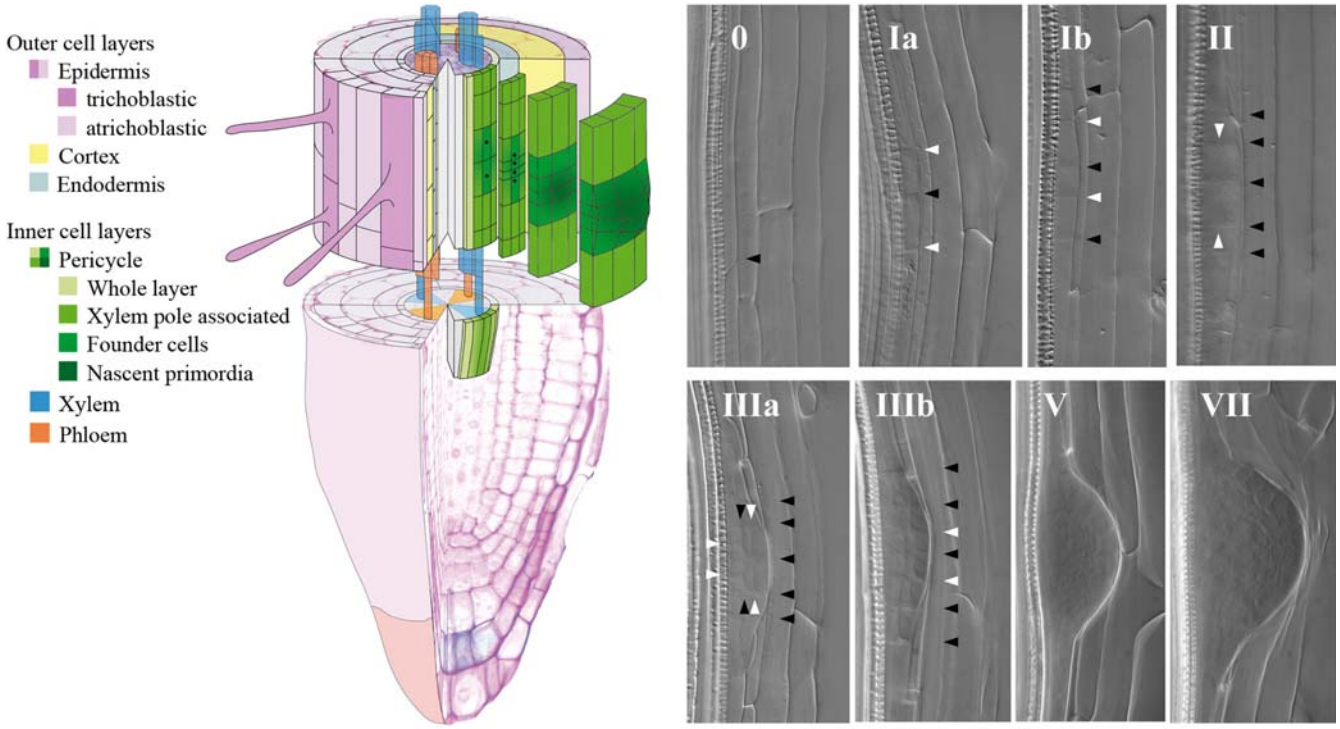

Fig. 5 Lateral root initiation in *Arabidopsis thaliana*. Left: Schematic representation of LR initiation. Lateral roots originate from pericycle cells adjacent to the xylem poles. Right: Different developmental stages during LR initiation. Black arrows indicate existing cell walls; white arrows indicate new cell divisions. Numbers refer to stages described in Malamy and Benfey (1997).

stage VI, the LR primordium begins its transformation from a layered primordium to a well-organised structure with a cellular pattern that mimics that of a mature PR tip. The four central cells divide periclinally by which the columella root cap is created, and as a result of a periclinal division of the second outer layer, three outer cell layers are laid down corresponding to epidermis, cortex and endodermis. The cells in the core of the LR primordium elongate, and obtain the elongated shape characteristic of provascular elements (Malamy and Benfey 1997). From stage V on, the auxin gradient at the primordium tip is established by combined action of differentially expressed and localised PIN proteins, mirroring the PIN localisation pattern of the PR. At stage VII, the primordium enlarges and is about to emerge from the parent root. LR emergence could be regarded as analogous to germination, as in both cases (LR and PR) once the organisation is established, there is a period during which growth is driven primarily by cell expansion. After this period, the meristem is activated and the newly autonomous root begins to grow via asymmetric divisions of the root stem cells. Thus morphologically and physiologically, the initiation, development, and emergence of the LR primordium and the subsequent activation of the LR meristem appear to be highly similar with the developmental mechanisms of embryogenesis.

Nonetheless the functional redundancy within the PIN family, single *pin* mutants already showed significant changes in the number of initiated LRs as well as a retarded emergence stage (Benkova *et al*. 2003). Auxin-treated multiple *pin* mutants were not able anymore to develop an LR primordium, instead these mutant seedlings produced a multi-layered pericycle without any trace of primordium formation. The same effects could be observed in wild-type seedlings treated with auxin transport inhibitors or in weak *gnom* mutants (Geldner *et al*. 2004), demonstrating that PIN-mediated auxin efflux is essential for primordium development.

Auxin signalling during lateral root formation

Over the years, it has been shown that mutations in auxin signalling affect LR formation (for review see Fukaki *et al*. 2005b). From the specification of the pericycle founder cell on, members of the Aux/IAA and ARF family translate the auxin accumulation into the expression of auxin-responsive genes. Among all reported genes of which the mutants show altered LR formation, *SLR* (*IAA14*), *ARF7*, and *ARF19* have been shown to play a prominent role during LR initiation (Fukaki *et al*. 2002; Okushima *et al*. 2005). *IAA14* is expressed in the pericycle and a dominant mutant version of the IAA14 protein has the ability to constitutively block LR initiation. Furthermore, IAA14 was shown to interact with ARF7 and ARF19 in a yeast two-hybrid screen (Fukaki *et al*. 2005a) and consistently the double mutant *arf7arf19* displays the same phenotype as *slr* (i.e. no LR formation) (Okushima *et al*. 2005a). These results indicate that SLR and ARF7/ARF19 mediate together the auxin-responsive gene transcription during LR initiation. Interestingly this auxin-signalling event is highly similar to the central role of BDL/IAA12 and MP/ARF5 during hypophysis formation. In this context, one can question whether the different Aux/IAA-ARF interactions really differ in function and do not solely rely on transcriptional regulation. Apparently, the specificity of the cellular auxin response is indeed dependent on the expression profiles of the *ARF* and *Aux/IAA* genes because ectopic expression of stabilised IAA14/SLR during embryogenesis, results in *mp*- and *bdl*-like embryos (H. Fukaki, pers. comm.). However, Weijers *et al*. (2005) could show that optimised pairs of interacting ARF and Aux/IAA proteins increase the specificity of the response.

Patterning during lateral root formation

The initiation of LRs is marked by specific cell divisions in the pericycle. However, although auxin stimulates the cell cycle, the formation of a new meristem is far more complex than a simple activation of the cell cycle machinery in the pericycle founder cells. Overexpression of *CYCD3;1* (*CYCD3;1*^{OE}) in the non-LR forming *slr* mutant, did not result in LR formation (Vanneste *et al*. 2005). Although, in some regions pericycle cells were shorter than wild-type pericycle cells, indicative for an extra round of cell division, the divided pericycle cells were found in long stretches differing fundamentally from normal LR initiation sites where

the region of cell division is much more restricted to a small group of cells. Furthermore no stage II primordia could be observed in the *CYCD3;1*^{OE} x *slr* roots. Thus, although auxin-induced cell division of the pericyle founder cells could be complemented in the *CYCD3;1*^{OE} x *slr* roots, additional auxin-dependent mechanisms are required for normal LR formation. Unfortunately not so much is known about cell specification during LR development. Nevertheless, it is likely that LR formation and embryogenesis share, besides the importance of auxin, comparable mechanisms for pattern formation. *SCR* expression could be demonstrated from a stage II LR primordium on (Malamy and Benfey 1997) and consistent with this, LRs of the radial pattern mutants *scr* and *shr* show the same patterning defects as during embryogenesis (Scheres *et al*. 1995). The QC-specifying and auxin-inducible *PLT* genes are ex-pressed already in the pericycle founder cells (Aida *et al*. 2004). In the *CYCD3;1*^{OE} x *slr* roots, *PLT1* expression was highly reduced, again highlighting the functionality of a SLR-dependent auxin signalling to establish a correctly patterned LR primordium (Vanneste *et al*. 2005). Furthermore, mutations in the *HOBBIT* gene lead to short LRs that display the same patterning defects as the PR (Willemsen *et al*. 1998), indicating that LR pattern formation can be seen as a replay of embryonic pattern formation.

Priming of pericycle founder cells occurs in primary root meristem

Since long, LRs have been considered to develop from newly formed meristems initiated from differentiated pericycle cells (Laskowski *et al*. 1995; Malamy and Benfey 1997). However the onset of LR formation from apparently fully differentiated cells is in sharp contrast to the embryogenic origin of the PR and has been a point of controversy for many root biologists. In the last years, experimental evidence is accumulating that argues against this dedifferentiation concept and instead stands up for a meristematic character for the pericycle cells (Dubrovsky *et al*. 2000; Beeckman *et al*. 2001; Casimiro *et al*. 2003). In an attempt to get insight into the pre-mitotic events of LR initiation, De Smet *et al*. (2007) focussed on the basal meristem, a zone between the meristem and the elongation zone. As mentioned above, the basal meristem has been proposed to recycle auxin coming from the root tip via the root cap (Blilou *et al*. 2005). De Smet *et al*. (2007) could demonstrate that the auxin-responsive DR5 promoter shows a rhythmic expression in the basal meristem with a periodicity that matches the rate of LR initiation. A model was proposed in which the pericycle cells, which later on will be activated in the differentiation zone to form LRs, are already triggered in the basal meristem. Furthermore this priming correlates with the gravitropic response-mediated waving of the PR which in turn is controlled by AUX1-mediated basipetal auxin transport. Consistently the left-right altering pattern of the LRs is completely disturbed in the *aux1* mutant. De Smet *et al*. (2007) could also show that the priming of pericycle founder cells is independent of IAA14/SLR, opening the quest for the Aux/IAA-ARF interacting partners necessary for the very first checkpoint towards LR formation.

Evidence for a pre-initiation of the pericyle cells in the PR meristem was also delivered by Horiguchi *et al*. (2003). The mutant *rfc3-1* (regulator of fatty acid composition) forms normal LRs but when germinating under a high sucrose condition (3% sucrose) the LR primordia show severe patterning defects from stage VI on. These LR primordia show no columella root cap initial activity, lack a structurally distinct QC and, after emergence, the top portion of the LR terminally differentiates into root hairs. *RFC3* encodes a plastid protein with weak homology to prokaryotic ribosomal subunit S6 (Horiguchi *et al*. 2003). Interestingly, Horiguchi *et al*. (2003) found that the sucrose concentration in which the PR is growing determines the patterning of LR primordia, suggesting the existence of a sucrose-sensitive priming mechanism in the RAM which is essential for LR patterning.

Shoot-borne root formation

In monocots, the PR appears to be essential for the growth at the seedling stage only. At later stages of development, the growth of the PR usually stops and post-embryonic shoot-borne roots start to develop and gradually determine the morphology of the fibrous root system (**Fig. 1**) (Feix *et al*. 2002). The shoot-borne crown roots are formed in variable numbers at underground stem nodes and initiate at the inner cell layer of the nodes. When formed from the above-ground nodes, such roots are designated as brace roots (Feix *et al*. 2002). In dicotyledonous plants, such as *Arabidopsis*, shoot-borne root formation is rare but can occasionally occur in hypocotyls or stems in response to exogenous factors like wounding or darkness. Since these roots are not developmentally regulated, they are referred to as adventitious instead of shoot-borne roots.

As mentioned above, auxins are plant hormones that regulate almost every aspect of plant growth and development including shoot-borne root formation. As a consequence, mutants impaired in auxin transport or signalling affect shoot-borne root formation as well. *OsPIN1* RNAi transgenic plants show a highly decreased crown root emergence and development, which was similar to the phenotype of NPA-treated wild-type plants (Xu *et al*. 2005). Consistently, *OsPINOID*-overexpression lines are characterized by a delayed shoot-borne root development (Morita and Kyozuka 2007). The rice genome contains 24 Aux/IAA genes and transgenic plants overexpressing a stabilized form of OsIAA3 showed typical auxin-insensitive phenotypes as a reduced crown and LR formation as well as a reduced root length of seminal, crown and LRs (Nakamura *et al*. 2006). Furthermore, *crl1* (*crown rootless*) and *arl1* (*adventitious rootless*), both mutated in the auxin-inducible *CRL1* gene, are defective in shoot-borne root formation and *crl1* shows also a 70% reduction in LR formation (Inukai *et al*. 2005; Liu *et al*. 2005). *CRL1* encodes an AS2 (asymmetric leaves 2)/LOB (lateral organ boundaries) domain transcription factor and a detailed analysis revealed that CRL1 is a member of an early auxin-response family under direct control of an ARF in the auxin signalling pathway (Inukai *et al*. 2005). Since the auxin-inducible expression of *CRL1* is inhibited in *OsIAA3*-overexpressing plants (Nakamura *et al*. 2006), it is likely that OsIAA3 together with a still unidentified OsARF regulate auxin-inducible crown root formation through the LOB transcription factor CLR1. Interestingly also in maize, the LOB-domain transcription factors *RTCS* (rootless concerning crown and seminal roots) and *RTCL* (*RTCS-LIKE*), are auxin-responsive genes involved in shoot-borne root formation (Hetz *et al*. 1996; Taramino *et al*. 2007). Additionally, in *rtcs* and *rtcl* mutants seminal root formation is also compromised and the only root that remains unaffected is the PR (Hetz *et al*. 1996). It will be very interesting to further examine the putative role of the *LOB* genes in root development, since the family is represented in other plant species including *Arabidopsis* (Yang *et al*. 2006). Recently, Okushima *et al*. (2007) identified the homologues of CRL1 in *Arabidopsis*. *AtLBD16* and *AtLBD29* were described as being the direct regulatory targets of ARF7 and ARF19 during LR initiation. Overexpression of *AtLBD16* and *AtLBD29* rescues LR formation of the *arf7arf19* mutant and dominant repression of LBD16 activity inhibits LR formation and auxin-mediated gene expression (Okushima *et al*. 2007).

As described above, rice *QHB* expression was observed during embryogenesis and crown root formation, prior to the morphological differentiation of the root. However, different *QHB* expression patterns were detected between primary and crown root formation during development of the respective root meristems in rice. This might suggest that cell-fate determination of the QC may be controlled by different mechanisms depending on the root type (Kamiya *et al*. 2003).

Several other mutants with a reduced number of shoot-borne roots have been identified over the last years (*rt1*: Jenkins *et al*. 1930; *crl2*: Inukai *et al*. 2001) but the corresponding genes are not identified until now.

Lateral root formation in monocotyledonous plants

As in dicotyledonous plants, in monocots LRs initiate from pericycle cells but, in contrast to dicots, initiation happens preferentially at the phloem poles. Also in contrast to dicots, the endodermal cells are involved by forming the epidermis and columella of the newly formed LRs (Bell and McCully 1970; Feix *et al*. 2002). LRs are formed on all root types in maize and rice, having a great influence on the architecture of the root system. Interestingly, characterisation of two maize mutants suggests that LR initiation in embryonic and post-embryonic roots are differently regulated. Both the *lateral rootless* (*lrt1*) (Hochholdinger and Feix 1998) and the *rootless with undetectable meristems* (*rum1*) (Woll *et al*. 2005) mutants are completely deficient in the initiation of LRs from the embryonic primary and seminal roots, while the shoot-borne root system shows normal LR formation. Additionally, *lrt1* lacks crown roots at the first node and *rum1* is deficient in embryonic seminal root initiation. A detailed analysis revealed that the *rum1* mutation leads to a reduced polar auxin transport in the PR but also to a reduced sensitivity of the pericycle towards auxin, suggesting a pleiotropic regulatory function for RUM1 in respect to auxin action (Woll *et al*. 2005). The LR-deficient *slr* mutant also shows a reduced auxin sensitivity and a reduction in auxin transport in *Arabidopsi*s. Therefore one could hypothesise that RUM1 could be involved in the primary auxin response in maize and would therefore represent the acting representative for the *Arabidopsis* IAA14. However since *rum1* is a loss-of-function mutation, RUM1 is most likely not a member of the Aux/IAA family.

Also in rice, auxin-sensitivity mutants were isolated. *arm1* and *arm2* were identified as being resistant to the synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D) and show a variety of morphological defects, including reduced LR formation (Chhun *et al*. 2003). Recently, Wang *et al*. (2006) isolated a novel lateral-rootless mutant in rice, *lrt2*, which is resistant to 2,4-D but also to NAA, IAA and IBA. Furthermore, the complete lack of LRs could not be rescued by exogenous auxin and molecular comparison with the *Arabidopsis* lateral rootless mutant *slr/iaa14*, led Wang *et al*. (2006) to hypothesise that the *LRT1* gene is a novel gene required for auxin-mediated LR formation that has not been reported in *Arabidopsis* yet.

IS POST-EMBRYONIC ROOT FORMATION IDENTICAL TO EMBRYONIC ROOT FORMATION?

Both developmentally and genetically, the similarities between the PR and LRs are striking. An asymmetric division controlled by efflux-dependent auxin transport initiates the process and a subsequent controlled cell division and patterning program establishes a highly organised structure where later on the meristematic initials are set aside. The patterning genes *PLT*, *SCR* and *SHR*, responsible for the embryonic root stem cell niche formation, are also expressed during the early stages of LR development and mutants show the same patterning defects in both root meristems. Moreover, these mechanisms seem to be at least partially conserved in monocotyledonous plants. This could be shown by a detailed expression analysis of *SCR* and *SHR* in maize and rice and by a complete rescue of the *Arabidopsis* patterning defects with the maize *SCR* gene.

However, it seems that there are differences between embryonic and post-embryonic roots. Overexpression of the *Arabidopsis* serine-threonine kinase *PINOID* induces a basal-to-apical shift of PIN localisation, which results in a reduced auxin accumulation in the PR tip, thereby causing the loss of stem cell identity and eventually the terminal differentiation of the RAM (Benjamins *et al*. 2001; Friml *et al*. 2004). The basal-to-apical shift of the PIN proteins could also be observed in LR meristems, however the latter remained functional, showed normal patterning and did not collapse. The reason for these differences is still unclear. It could be due to increased auxin biosynthesis in LR meristems or alternatively, to the different origin of PR and LR meristems (Friml *et al*. 2004). *mp* seedlings that lack a PR due to hypophysis misspecification, can be stimulated by injuring to form roots from the hypocotyl. Surprisingly, these adventitious roots develop normally (Przemeck *et al*. 1996; Hardtke and Berleth 1998). In maize, the characterisation of the *lrt1* and *rum1* mutants indicated that LR initiation in embryonic and post-embryonic roots are differently regulated (Hochholdinger and Feix 1998; Woll *et al*. 2005), which in turn is indicative for the existence of alternative root developing programs in embryonic and post-embryonic roots.

PERSPECTIVES OF GENOME-WIDE ANALYSIS OF ROOT FORMATION GENES

Over the last years, tremendous efforts have been made to analyse all aspects of root development in a genome-wide perspective. Nowadays cell sorting and laser capture microdissection (LCM) allow the isolation of specific root cell types. When combining these isolation strategies with micro-array analysis, very reliable expression maps can be obtained for specific cell types during root development. LCM together with micro-array analysis was applied to isolate and profile different domains of embryos during the globular, heart and torpedo stages (Spencer *et al*. 2007). Using cell sorting on five GFP-expressing lines representing specific cell layers Birnbaum *et al*. (2003) succeeded in making a gene expression map of the *Arabidopis* root. A few years later, the paper was followed by a transcript profiling of *Arabidopsis* QC cells (Nawy *et al*. 2005). Although transcript profiling using the LR-deficient *arf7arf19* and *slr* mutants identified specific subsets of genes involved in LR development (Okushima *et al*. 2005; Vanneste *et al*. 2005), such an expression map is until now not available for LRs. By comparing available and future expression data, we will increase our understanding of parallel mechanisms between embryonic and post-embryonic root development.

Also in maize, LCM has been used in order to study differential gene expression between epidermal cells and vascular tissue (Nakazono *et al*. 2003) and between pericycle transcripts of wild-type and that of the LR initiation defective *rum1* mutant (Woll *et al*. 2005). More recently, Dembinsky *et al*. (2007) compared gene expression and protein accumulation in cell-cycle-competent PR pericycle cells of maize prior to their first division and LR initiation.

In this review we have summarized the molecular networks controlling root development in monocotyledonous and dicotyledonous plants. Although the huge morphological differences between both root systems, it can be concluded that the basic molecular mechanisms controlling root development are far more conserved than expected. Further transcriptome-wide analyses of specific mutants on a cell-type-specific level will ultimately uncover the degree of conservation of the functional networks between monocots and dicotyledonous plants.

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