Planta (1998) 205: 483–491

Planta

Review

Cell fate and cell differentiation status in the *Arabidopsis* root

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Received: 5 November 1997 / Accepted: 14 January 1998

Abstract. Post-embryonic development in plants is mainly achieved by its meristems. Within the *Arabidopsis* root meristem, both the fate and origin of its cells can be predicted with high accuracy. Mutants defective in the determination of root cell fates show that the corresponding genes are first required during embryogenesis. The sub-specification of cell fates, such as during epidermal root hair formation, involves transcription factors and phytohormones. In the *Arabidopsis* root, initial cell fate specification events must be followed by position-dependent reinforcement of cellular identity. A major question remains as to whether the signals that are involved in initiating the cellular pattern are the same or different from the signals used to reinforce it. The integrity of the root meristem is kept by balancing cell proliferation and cell differentiation, and differentiation-inhibiting signals originating from the quiescent centre are involved.

Key words: *Arabidopsis* – Cell differentiation – Cell fate – Root meristem

Introduction

During embryogenesis of higher organisms, cell types are specified in a specific arrangement during the process of pattern formation. In plants, patterning is not limited to embryogenesis but continues during the adult life cycle as a result of the activity of meristems. Two meristems form during embryogenesis at opposite sides of the apical-basal axis: the shoot apical meristem and the root meristem. In the higher-plant embryo, particular organs are positioned between the meristems: the cotyledons, the hypocotyl and the embryonic root. Perpendicular to the apical-basal axis, different cell types are laid down in concentric layers to form the radial pattern.

Upon germination, new cells and organs arise from the meristems to elaborate the pre-existing cellular pattern of the embryo. The continuation of an embryonic pre-pattern by the meristems is more clearly seen in the root than in the shoot meristem, because the root meristem lacks the complexity of lateral appendage formation.

Here we discuss two issues in plant developmental biology that are currently under intense investigation. We take examples from *Arabidopsis* root development as this root has proven to be an elegant model system in which to study plant developmental processes (reviewed by Scheres and Wolkenfelt 1997).

The first issue is the mechanisms by which plant cells can acquire and maintain distinct identities. Due to the uncomplicated cellular organization of the *Arabidopsis* root, in which the developmental history of every cell is known, cell fate specification can be studied at a single cell level (see Figs. 1, 2). Mutants defective in setting up the pattern of cell types in the root are being isolated and their characterization may shed a light on the processes of cell fate specification in plants. Furthermore, in-vivo manipulations involving laser ablations and microinjections, are beginning to provide clues as to mechanisms involved in cell fate specification.

A second issue of current interest is that, within the meristems, cell proliferation occurs in a co-ordinated balance with cell differentiation to maintain a reservoir of undifferentiated cells. Until recently, not much was known about the control of the equilibrium of cell differentiation versus cell proliferation, but recent data from analysis of both root and shoot meristems, provide new insights into this process. In this review, we will highlight data that illustrate new insights into control of cell fate and cell differentiation. Reviews with additional perspectives on root pattern formation and morphogenesis have been recently published elsewhere (Dolan 1996; Malamy and Benfey 1997a; Schiefelbein et al. 1997).

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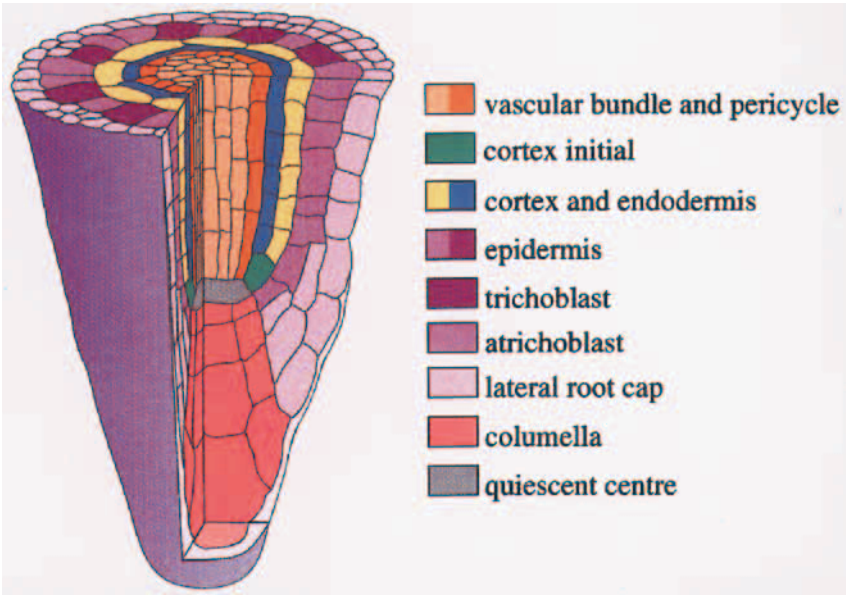


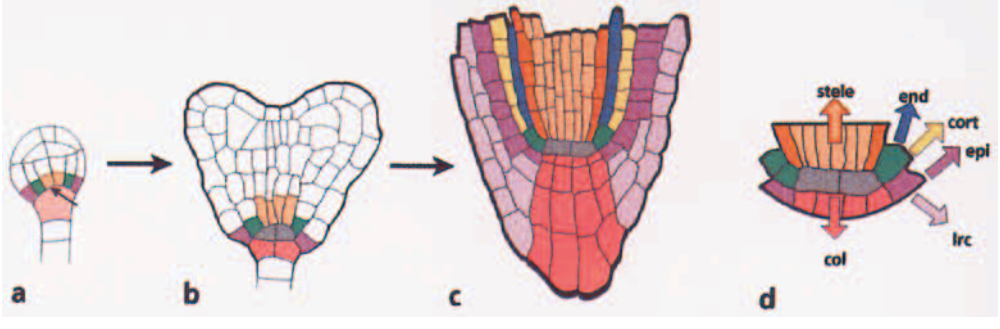
Fig. 1. Schematic representation of the *Arabidopsis* root

Cellular organization of the *Arabidopsis* root

The *Arabidopsis* root has an uncomplicated cellular organization (Fig. 1; see also Dolan et al. 1993). Within each tissue layer is a low, almost constant, number of cells as viewed in a transverse section. A small vascular bundle, comprising two phloem and two xylem poles in a diarch arrangement, is surrounded by single layers of pericycle, endodermis, cortical parenchyma (cortex hereafter) and epidermis. At the basal end of the root, lateral root cap layers envelop the epidermis. In longitudinal view, the lateral root cap forms continuous files with the columella root cap (Figs. 1, 2c). Files of the different cell types are extended in the meristem by the activity of mitotically active initial cells and their daughters (Fig. 2d). The initials are located basally in the root and are considered to be stem-cell-like cells. They generate new initials which remain in a less differentiated state, and daughter cells which further differentiate. Separate sets of initials are present to generate the vascular bundle cells, the pericycle cells and the columella cells. The epidermal initials generate both the epidermis and the lateral root cap, and the cortical initials form the cortex and the endodermis. All initial cells contact the four mitotically inactive cells of the quiescent centre (Fig. 2d). The orientation of cell divisions in the *Arabidopsis* root meristem is highly invariant and this maintains the overall organization of the root. However, in roots older than a week, differences in cell division patterns cause variation in the cellular organization of the root (Baum and Rost 1996; Rost et al. 1996). First, periclinal divisions in the cortical initials result in the formation of separate initials for cortex and endodermis. Second, the quiescent centre can be activated to divide to form extra cortical cells.

Daughter cells of the initials are gradually displaced from the meristem and they differentiate into specific cell types during that process. For example, in epidermal cells, differentiation leads to the formation of a circumferential pattern consisting of root-hair-bearing and non-hair-bearing cells (Fig. 1; recently reviewed by Dolan 1996, Schiefelbein et al. 1997). However, during the course of cell differentiation, pericycle cells can be reset to initiate new organ formation. These cells re-enter mitosis, resulting in the formation of lateral roots with a similar pattern as the primary root (Dolan et al. 1993).

Fig. 2a–d. Fate map of the *Arabidopsis* root. **a** Globular stage embryo (*arrow*: first division of the zygote that has generated an apical and a basal cell. **b** Heart-stage embryo; all cells of the incipient root meristem are present, **c** Seedling root, **d** Blow-up of central region in root meristem shown in **c**. Initial cells for all the different cell types surround the quiescent centre. *cort*, cortex; *epid*, epidermis; *lrc*, lateral root cap, *col*, columella



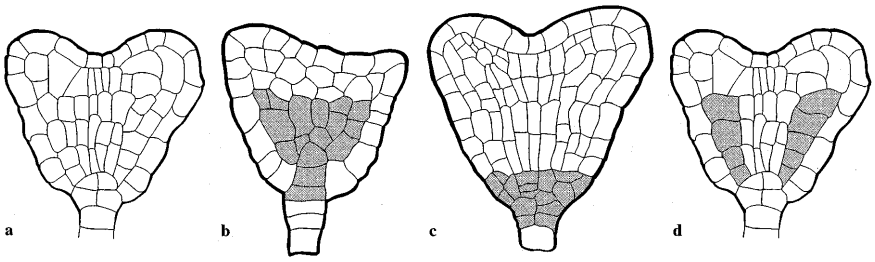


Fig. 3a–d. Schematic representations of heart-stage embryos. **a** Wild type; **b** *monopteros*, a central pile of cells runs in continuity with the suspensor; **c** *hobbit*, divisions in the hypophyseal cell derivatives and root meristematic initials are defective; **d** *scarecrow*/*shortroot*, in both mutants no periclinal divisions in the ground tissue occur. (**b** redrawn from Przemeck et al. 1996; **c** redrawn from Willemsen et al. 1998; **a**, **b**, **d**: early heart stages; **c**, late heart stage). The affected regions in each mutant are shaded. These do not necessarily represent gene expression domains

In conclusion, the *Arabidopsis* root has a rather uncomplicated cellular organization, and initial cells and their daughters perpetuate the existing pattern by ordered cell divisions and subsequent differentiation of daughters.

Setting up the pattern

The cellular organization of the root is established during embryogenesis. In *Arabidopsis*, the first division of the zygote generates an apical and a basal cell (Fig. 2a, arrow). The root is formed from derivatives of both these cells. The ontogeny of the *Arabidopsis* root could be described at the cellular level through the analysis of the progeny of genetically marked embryonic cells (Scheres et al. 1994).

The apical-basal pattern within the root results from the ordered development of both apical and basal cell derivatives. The upper derivative of the basal cell, the hypophysis, generates the quiescent centre and the columella root cap initials (Fig. 2b). Apical-cell derivatives make the remainder of the root and root meristem. The cells contacting the hypophysis generate the initials of the root meristem during the heart stage of embryogenesis. The most apical part of the root that connects to the hypocotyl, the collet or embryonic root, is produced by more-apical cells in the embryo.

The radial organization of the root is generated within a group of apical-cell-derived cells at the globular stage of embryogenesis (Fig. 2a). At that stage, cells for the protoderm, ground tissue and procambium (later forming epidermis, cortex/endodermis and vascular bundle, respectively) become separated (Fig. 2b). The mitotic activity of the root meristem initials extends these separate tissue layers basally, from late heart stage onwards.

Hence, an invariant group of embryonic cells participates in the formation of the apical-basal and radial pattern in the root. The separation of different root cell fates occurs during early embryogenesis. It is noteworthy that the separation of the root and hypocotyl occurs much later during embryonic development and displays some variability: cells around the border can be incorporated either in the root or in the hypocotyl. Their fate is thus dependent on late positional cues.

Several mutant screens have been set up to identify genes involved in determination of cell fates of apical-basal, radial and circumferential patterning elements of the root. Mutants were isolated on basis of their seedling phenotype (Schiefelbein and Somerville 1990; Benfey et al. 1993; Berleth and Jürgens 1993; Galway et al. 1994; Scheres et al. 1995; Schneider et al. 1997; Willemsen et al. 1998). Below, we describe those that have been characterized in some detail.

Apical-basal pattern formation. A number of mutants have been isolated which lack pattern elements in the apical-basal axis of the embryo (Mayer et al. 1991; Scheres et al. 1996). Mutations in the *MONOPTEROS* (*MP*) gene lead to seedlings lacking both root and hypocotyl (Berleth and Jürgens 1993). The defect in *mp* mutants is evident as early as the octant stage of embryogenesis. At the triangular stage, cells of both the lower tier and the hypophysis divide aberrantly. The inner cells of the lower tier, which make the vascular cells of root and hypocotyl, fail to produce elongated cell files. Furthermore, a pile of cells in the centre forms by horizontal divisions, running in continuity with the suspensor (Fig. 3b).

The capacity of *mp* mutants to make adventitious roots was used to study the post-embryonic requirements for the *MP* gene product. The *mp* plants can make largely normal apical structures. All organs, however, display defective vascular strands that are disrupted (Przemeck et al. 1996). Furthermore, auxin transport capacity in inflorescence axes is impaired. These observations led us to the conclusion that MP, rather than specifying apical-basal pattern elements, promotes cell axialization and cell file formation which is important for both embryonic axis formation and vascular system development.

Cell fate determination along the apical-basal axis also involves the *HBT* gene. The *hbt* mutants have cotyledons, a hypocotyl and a small embryonic root. However, no specification of the meristematic root occurs (Willemsen et al. 1998). Lateral and adventitious roots also form abnormally in *hbt*. The mutational defect first becomes apparent around the quadrant stage of embryogenesis. From that stage onwards, atypical divisions occur in the hypophyseal progenitor cell (Fig. 3c). This defect correlates precisely with the absence of an anatomically recognizable quiescent centre

and the lack of columella root cap cell specification in *hbt* seedlings. After the heart stage of embryogenesis, the divisions of the cells occupying the position of the initial cells in the wild type are strongly reduced or completely absent in *hbt* mutants (Fig. 3c). Furthermore, in the region where normally the lateral root cap is formed, divisions are defective. These defects lead to seedlings without a functional root meristem. The *HBT* gene could thus be involved in specifying the hypophyseal cell derivatives, the root meristematic initials and the lateral root cap. Alternatively, *HBT* could be involved primarily in specifying the hypophyseal cell. Subsequently, *HBT*-expressing cells could signal to the directly contacting apical cells to initiate meristematic mitotic initial activity and lateral root cap specification. In the first case, *HBT* would be functional in the complete root meristem. In the latter, *HBT* would be functional in the hypophyseal cell derivatives only. The *hbt* seedlings are also impaired in cell expansion and epidermal cell development in regions not affected in the embryo, suggesting additional post-embryonic requirements for the HBT gene.

Radial pattern formation. Several mutants have been described lacking radial pattern elements in the root (Benfey et al. 1993; Scheres et al. 1995). Both *scarecrow* (*scr*) and *shortroot* (*shr*) lack a layer of the ground meristem (Fig. 3d). The *shr* mutants lack an endodermis whereas the remaining ground-tissue layer in *scr* mutants expresses both cortical and endodermal markers (Scheres et al. 1995; Di Laurenzio et al. 1996). Both mutant phenotypes are first manifested at the heart stage of embryogenesis where the periclinal division that doubles the ground meristem does not occur in the root or in the hypocotyl (Fig. 3d). To determine whether the *shr* and *scr* mutants are primarily disturbed in cell division in the ground tissue, or primarily in fate specifications of the ground tissue, double mutants with *fass* were made (Scheres et al. 1995). The *fass* mutants display additional cell divisions in all cell layers (Torres-Ruiz and Jürgens 1994). Double mutants of *shr* and *fass* have more ground-tissue layers. However, an endodermis-specific cell layer could not be restored. Considering these observations, it was concluded that SHR primarily specifies endodermal cell fate. In contrast, *scr* and *fass* double mutants have a single endodermal layer surrounded by multiple layers lacking endodermal attributes. Apparently, SCR is involved in controlling the periclinal division in the ground meristem that leads to separate cortical and endodermal cell layers. Recently, the *SCR* gene has been cloned (Di Laurenzio et al. 1996). It encodes a novel putative transcription factor, and it is not known how the gene product controls cell division. It is likely that other, SCR-dependent, genes are involved in the process that ultimately leads to the periclinal division in the ground meristem. In-situ hybridization and an enhancer-trap insertion upstream of the *SCR* gene reveal that *SCR* is expressed in the cortical initial and in all endodermal cells. This contrasts with the assumption that SCR regulates the asymmetric division in the cortical initial only, to separate cortex and endodermis. More work is needed to determine the functional relevance of the endodermal expression of *SCR*. It is possible that *SCR* is required both for the division in the cortical initials to separate cortex and endodermis, and for endodermal fate specification or maintenance.

A mutation in the *WOODEN LEG* (*WOL*) gene results in seedlings with fewer vascular cells in root and hypocotyl, without phloem specification (Scheres et al. 1995). Enhancing the amount of cells (in a *fass* background) restores phloem formation. This indicates that the *wol* mutant primarily interferes with cell division, with a lack of specific cell fates as a secondary consequence, rather than being involved primarily in cell fate specification. The amount of cells available seems to be critical at the period during which cell types are specified. In *wol* mutants, the formation of xylem cells may use up all the available cells and thus no phloem forms. This 'first-come-first-served' mechanism was also suggested for the action of WUSCHEL in floral meristems (Laux et al. 1996). The *WUSCHEL* gene is required for the identity of the central zone in shoot and floral meristems. In *wuschel* mutant flowers, fewer than the normal number of cells are present, and third- and fourth-whorl organs are not formed.

Taken together, mutant screens based solely on seedling phenotypes have revealed a limited number of mutants affecting the radial organization in the root, and these confirm the notion that the cellular pattern of the root is set up in the embryo. The screens, however, are based on the overall appearance of the root (e.g. root length). Some mutants defective in the radial organization of the root may be very subtle, and have no effects on overall root growth. Furthermore, these screens fail to detect a conceivable class of embryo-lethal pattern mutants. Additional screens using β-glucuronidase or Green Fluorescent Protein coupled to tissue specific promoters, to mark the presence of cell types and hence enable genetic screens for ectopic presence or loss of these cell types, might unravel more players in the process of radical pattern formation in the future.

Circumferential pattern formation. Once outside the meristem, cells further elongate and differentiate. Epidermal cells have the ability to form root hairs. In the *Arabidopsis* seedling root, files of hair-bearing (trichoblast) cells alternate with files of hairless (atrichoblast) cells. The trichoblast cells are located over the cell wall clefts between two underlying cortical cells whereas non-hair cells only contact a single cortical cell (Dolan et al. 1994). It is thought that the occurrence of radial walls in underlying cortical cells determines, at least in part, epidermal cell fate (see below). The difference between trichoblast cells and atrichoblast cells is already visible prior to the outgrowth of the hair. Trichoblasts are more elongated, have a more densely stained cytoplasm and have a delayed vacuolation (Galway et al. 1994).

Mutant analyses have identified genes involved in root hair formation (Table 1). The mutants *transparent testa glabra* (*ttg*), *glabra2* (*gl2*), *ectopic root hair1* (*erh1*), *ectopic root hair2* (*erh2*) and *ectopic root hair3* (*erh3*) have root hairs on almost all epidermal cells (Galway

et al. 1994; Masucci et al. 1996; Schneider et al. 1997). In *ttg*, *erh1* and *erh3* mutants, all morphological differences between atrichoblast and trichoblast cells are lost, whereas in *gl2* and *erh2* mutants some morphological differences between these two cell types still exist. *GL2* encodes a putative homeodomain-containing transcription factor and is expressed in all atrichoblasts, except in the epidermal initials and their first and second daughters (Rerie et al. 1994; Masucci et al. 1996). In *ttg* mutants, *GL2* expression is strongly reduced, suggesting that in wild-type *TTG* is a positive regulator of *GL2* (Di Christina et al. 1996).

The *caprice* (cpc) mutants have fewer root hairs than the wild type. Recently, the *CPC* gene was cloned (Wada et al. 1997). It encodes a protein with a Myb-like DNA-binding domain. Double mutant combinations with *GL2*

Table 1. Mutants defective in cell fate specification in *Arabidopsis* roots discussed in this review. WT, wild type

Gene	Mutant phenotype	Proposed WT function	Protein encodes	Reference
Pattern formation				
monopteros (*mp*)	No root and hypocotyl; vascularization defects	Cell axialization		Berleth and Jürgens 1993
hobbit (*hbt*)	Misspecification of hypophyseal cell; no meristem activity	Specification of root meristem		Willemsen et al. 1998
shortroot (*shr*)	No endodermis	Endodermal cell fate		Scheres et al. 1995
scarecrow (*scr*)	No separation into cortex and endodermis	Asymmetric cell division in ground tissue	Transcription factor (bZIP domain)	Di Laurenzio et al. 1996
wooden leg (*wol*)	Fewer vascular cells (no phloem)	Cell divisions in vascular bundle		Scheres et al. 1995
Root hairs				
constitutive triple response1 (*ctr1*)	Excessive root hairs	Negative regulator of root hair development and ethylene signal transduction	Raf kinase	Kieber et al. 1993; Dolan et al. 1994
transparent testa glabra (*ttg*)	Excessive root hairs	Negative regulator of root hair development		Galway et al. 1994
glabra2 (*gl2*)	Excessive root hairs	Negative regulator of root hari development	Transcription factor (homeodomain)	Masucci et al. 1996
ectopic root hair1 (*erh1*)	Excessive root hairs	Negative regulator of root hair development		Schneider et al. 1997
ectopic root hair2 (*erh2* = *pom1*)	Excessive root hairs	Negative regulator of root hair development		Schneider et al. 1997
ectopic root hair3 (*erh3*)	Excessive root hairs	Negative regulator of root hair development		Schneider et al. 1997
root hair defective6 (*rhd6*)	Fewer root hairs	Promotes root hair formation		Masucci and Schiefelbein 1994
caprice (*cpc*)	Fewer root hairs	Positive regulator of root hair development	Transcription factor (Myb domain)	Wada et al. 1997
root hairless1 (*rhl1*)	No root hairs	Early acting activator of root hair fate		Schneider et al. 1997
root hairless2 (*rhl2*)	No root hairs	Early acting activator of root hair fate		Schneider et al. 1997
root hairless3 (*rhl3*)	No root hairs	Early acting activator of root hair fate		Schneider et al. 1997
Lateral roots				
aberrant lateral root formation3 (*alf3*)	Aborted lateral root primordia (arrested primary root)	Elevates IAA levels in primordium		Celenza et al. 1995
superroot (*sur1* = *alf1* = *hls1* = *rty*)	Multiple root primordia (primary root reduced elongation)	Modulates IAA levels		Boerjan et al. 1995; Celenza et al. 1995
aberrant lateral root formation4 (*alf4*)	No lateral root primordia	Sensor of \responder to IAA		Celenza et al. 1995

suggest that *CPC* may inhibit *GL2*. As the expression pattern of *CPC* was not determined, it is unknown in which epidermal cells *CPC* is active and thus whether it is possible that CPC can directly inhibit *GL2*.

Ethylene plays a role in root epidermal fate and promotes the formation of root hairs (Tanimoto et al. 1995; Masucci and Schiefelbein 1996). Germination of plants in the presence of 1-amino-1-cyclopropane carboxylate (ACC), an ethylene precursor, results in ectopic hair formation. Addition of 2-aminoethoxyvinyl glycine (AVG), an inhibitor of ACC formation, leads to a decrease in root hairs.

A number of mutations in the ethylene response pathway have been implicated in epidermal cell patterning. One of these genes, *CONSTITUTIVE TRIPLE RESPONSE1* (*CTR1*) encodes a RAF-like kinase and is proposed to negatively regulate ethylene signalling (Kieber et al. 1993). The *ctr1* mutants form root hairs on epidermal cells that are normally hairless (Dolan et al. 1994). Furthermore, *ROOT HAIR DEFECTIVE* (*RHD6*) mutations cause a strong reduction in root hair formation and the mutant phenotype can be suppressed by ACC (Masucci and Schiefelbein 1994). The *root hairless1* (*rhl1*), *root hairless2* (*rhl2*) and *root hairless3* (*rhl3*) mutants lack root hairs. Double mutant analysis and ethylene addition experiments indicate that the *RHL* genes act before *CTR1*, the *ERH* genes, ethylene and probably before *TTG* and *GL2* (Schneider et al. 1997). Together with the loss of morphological differences between trichoblasts and atrichoblasts in the mutants, these data suggest that the *RHL* genes play the earliest role in epidermal cell fate specification analyzed so far. The *rhl* mutants also have defects in shoot development which suggest that the corresponding gene products might have more than one role in plant development.

It was shown that the expression of *GL2* is not altered by the presence of ethylene or in a *rhd6* or *ctr1* mutant background (Masucci and Schiefelbein 1996). Double mutant analysis indicated that RHD6 is negatively regulated by, or acts separately from, TTG and GL2. These data may imply that TTG and GL2 act upstream of ethylene by negatively regulating the ethylene signalling pathway. It was proposed that the position-dependent pattern of epidermal cell types is controlled by TTG and GL2, which results in repression of the ethylene signal transduction pathway (Masucci and Schiefelbein 1996). In addition, epidermal cells may receive different amounts of ethylene, resulting in a differential activation of the ethylene signalling cascade (Dolan et al. 1994).

In conclusion, mutant analyses begin to shed some light on the processes of fate determination in the root, most notably in the case of epidermal sub-specification. However, these analyses do not reveal at this moment how the meristematic pattern is perpetuated and we are still far away from understanding cell specification at the molecular level.

Formation of lateral roots

How are distinct identities of cells acquired and elaborated, in non-embryogenic roots? Does pattern formation require the same signals in both primary and lateral roots? Lateral roots are formed after embryogenesis and their anatomy is highly similar to that of primary roots. Lateral roots arise from pericycle cells that re-enter mitosis. The addition of exogenous auxin dramatically increases the number of lateral roots (Celenza et al. 1995 and refs. therein). The *superroot* (*syn, aberrant lateral root formation1 syn hookless3*) mutants, which have an increased indole acetic acid (IAA) level, display a similar phenotype (Boerjan et al. 1995; Celenza et al. 1995). Moreover, auxin-resistant mutants have a reduced number of lateral roots (Hobbie and Estelle 1995; Timpte et al. 1995). Other mutants defective in lateral root formation seem to be insensitive to application of IAA. In *aberrant lateral root formation4* (*alf4*) mutants, lateral root formation is not stimulated by the addition of IAA or the introduction of mutants with elevated IAA levels in an *alf4* mutant background. Although it clearly serves an important role, the mechanism by which auxin induces lateral root formation is unknown. The *alf4* mutants fail to initiate lateral roots but the primary root develops normally (Celenza et al. 1995). On the other hand, *mp* mutants make normal secondary roots, indicating that MP is strictly required for root initiation in the embryo only (Berleth and Jürgens 1993).

Upon initiation, however, mutant analyses and the expression patterns of tissue specific markers suggest that similar mechanisms of pattern formation are used to further set up and elaborate the pattern in roots derived from different origins (Willemsen et al. 1998; Scheres et al. 1995; Malamy and Benfey 1997b). Mutations in *HBT*, *SHR*, *SCR* and *WOL* display the same defects in both primary and lateral roots (Willemsen et al. 1998; Scheres et al. 1995). In conclusion, it is likely that lateral root initiation is regulated differently from primary root initiation. However, once initiated, pattern formation appears to be regulated by a similar set of genes.

Lateral root primordia can, from early stages onwards, be excised and grown further (Laskowski et al. 1995). Furthermore, marker genes that are expressed early in lateral root formation appear to have comparable expression patterns in the embryo (compare Di Laurenzio et al. 1996 with Malamy and Benfey 1997b). Mutant screens based on altered expression patterns of marker genes in lateral roots are less laborious than screening for altered or missing expression patterns in embryos. Studying lateral roots may thus be a promising approach to enhance our knowledge on cell fate specification during formation of the less accessible primary root.

Position determines cell fate in the root meristem

Because the root forms no lateral extensions within the meristem, one can clearly observe that once the

meristematic organization is established, the resulting pattern is continuously perpetuated. New cells are added to the root by the activity of initials and their daughters. How is the cellular pattern perpetuated? How do cells within the meristem know when and how to divide and into which cell type they should differentiate? The rigid clonal relationship between meristematic cells and their ancestors in the *Arabidopsis* root can be taken to indicate that cell fate determinants are distributed early, with no or only limited flexibility for switching fate at later stages. However, surgical experiments in various meristems showed that meristematic cells can be very flexible (reviewed by Steeves and Sussex 1989). Accordingly, laser ablations in the *Arabidopsis* root meristem showed that cells change fate upon changing position (van den Berg et al. 1995). Ablation of cells results in their shrinking and the available space becomes occupied by neighbouring cells. For instance, ablation of a cortical initial leads to the invasion of pericycle cells. These pericycle cells start producing cortex and endodermis in accordance with their new position. Isolation of a cortical initial from its daughters perturbs its specific differentiation, indicating that positional cues originate from older cells of their own cell type (van den Berg et al. 1995). Cells thus differentiate similarly to their more apical daughters. The molecular nature of these positional cues is thus far unknown and it is not clear how they are distributed.

Recently, the mapping of plasmodesmata and dye-loading experiments have indicated that plasmodesmata are preferentially located within files of the same cell type and not between different cell types (Oparka et al. 1994; T. Zhu, W. Lucas, and T.L. Rost, UCA Davis, USA. personal communications). It is thus well conceivable that positional signals are targeted via plasmodesmata. Support for this suggestion comes from fluorescent dye injections in the epidermis. The dye can move to other epidermal cells but not to cells of other cell types (Duckett et al. 1994). Additional evidence for the importance of plasmodesmatal transport comes from studies on fate determination in the shoot meristem. *KNOTTED-1* mRNA is localized in all cells of the shoot meristem except the outermost layer (L1 layer; Jackson et al. 1994). The KNOTTED-1 protein, however, is present in all cells including the L1 layer. Injection experiments with fluorescently labelled KNOTTED-1 protein and its mRNA showed that KNOTTED-1 can traffic between cells and that it facilitates the movement of *KNOTTED-1* mRNA to other cells (Lucas et al. 1995). Ablation experiments disturb plasmodesmatal connections, but the possibility of rapid re-formation of plasmodesmata in new neighbours does not preclude a role for plasmodesmatal transport in respecification events upon laser ablation.

Alternatively, cell fate could be determined by the selective depositing of signalling molecules in the cell wall. Laser ablations in the brown alga *Fucus* have shown that cell wall components can be instructive in cell fate decisions (Berger et al. 1994). The laser ablation experiments in the *Arabidopsis* root do not provide information about whether cell walls are instrumental in fate determination, since short-lived wall molecules may not persist in walls surrounding corpses of ablated cells. The identification and analysis of molecular components involved in signalling are required to determine whether positional cues guiding root cell fate are targeted via plasmodesmata or via the cell wall.

The laser ablation experiments show that the root meristem acts to copy the existing embryonic pattern, which can compensate for variations in meristematic cell division patterns. It is possible that the same signals that are active in embryogenic patterning, also function post-embryonically as continuous positional cues. Alternatively, different signals can be used in setting up and in perpetuating the cellular pattern.

The regulation of cell proliferation and differentiation

A proper balance between cell proliferation and cell differentiation in meristems is necessary to guarantee continuous development. If signals guiding cell fate resulted in progression of cell differentiation in the initial cells, a determined meristem would be the result. What keeps initial cells from differentiating? It is noteworthy that initials can not be considered as undifferentiated cells. Certain cell-type-specific promoter/enhancer trap constructs show expression in a complete tissue, including its initials (e.g. van den Berg et al. 1995). Therefore, the progression of differentiation should be regulated and not the maintenance of a group of completely undifferentiated cells.

Ablation of a quiescent-centre cell results in the progression of differentiation of contacting columella initials. These initials now express a marker that is normally only expressed in more-mature columella cells (van den Berg et al. 1997). In contacting cortical initials,

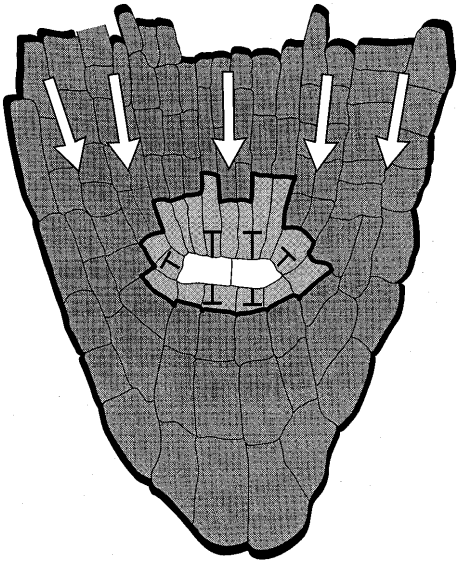


Fig. 4. A simple model representing two different regulatory signals within the root meristem. The quiescent centre inhibits differentiation of surrounding initials, whereas positional cues direct differentiation into different cell types

progression of differentiation was also observed after quiescent-centre ablation, resulting in the division of cortical initials in a 'daughter-specific' manner. Thus, the quiescent centre appears to inhibit the progression of differentiation of surrounding cells in the root (Fig. 4). Inhibition of differentiation only occurs in cells that contact the quiescent centre, which reveals that short-range or contact-dependent signals are involved in this process. It is noteworthy that all the initial cells contact the quiescent centre. In conclusion, the quiescent centre plays an important role in controlling the balance between cell proliferation and cell differentiation, although additional controls may be involved.

In the shoot meristem, mutants have been characterized that are disturbed in cell proliferation and/or differentiation. Mutations in the *CLAVATA1* and *CLAVATA3* genes alter the balance of cell proliferation versus cell differentiation (Clark et al. 1993, 1995). The *clavata* mutants have enlarged shoot and floral meristems caused by excessive accumulation of undifferentiated cells. The gene *CLAVATA1* encodes a putative receptor kinase and is expressed in the central undifferentiated cells and in a portion of cells contributing to organ formation (Clark et al. 1997). Studies on the *clavata* mutants have not yet revealed whether CLAVATA inhibits cell division of stem cells or whether it promotes incorporation of differentiated cells into organs.

Root meristems also contain a central zone of less differentiated cells. It will be interesting to find out whether *CLAVATA*-like genes play a role in the control of cell proliferation and cell differentiation in a similar manner in the root as in the shoot meristem.

Concluding remarks

Initiation of the cellular pattern during embryogenesis and perpetuation of this pattern during seedling development, and regulation of the balance between cell proliferation and cell differentiation are two important aspects of root development. Regarding the first aspect, several mutants have been identified with specific defects in the root cellular pattern. It turns out that the corresponding genes are first required during the early stages of embryo development, and they are required throughout the embryonic axis. Circumferential patterning seems to involve pre-patterned transcription factors (such as *GL2*), and phytohormones (most notably ethylene), which may be differentially allocated to or received by epidermal cells. Comparisons of mutant phenotypes and marker-gene expression patterns indicate that primary root formation probably involves the same mechanisms as secondary root formation. To construct a better genetic framework for cell specification in the *Arabidopsis* root, novel mutant screening strategies will be required. The use of markers for specific cell types in primary or lateral roots will be useful in these screens to identify novel genes involved in pattern formation.

Laser ablation experiments indicate that positional information continuously acts to reinforce the specification of newly added cells in the root meristem. Mutant analysis has not yet provided a genetic entrance in the nature of these positional signals. Future challenges will be to resolve both the nature of the positional cues and the mechanisms to receive these cues.

In the root meristem, signals derived from the quiescent centre keep the surrounding initial cells in a less differentiated state. It will be interesting to determine whether there is an interplay between quiescent-centre-derived signals inhibiting differentiation, and positional signals promoting cell-type-specific differentiation.

Molecular genetic and surgical approaches will ultimately lead to a more complete picture of how the *Arabidopsis* root develops. Although it is likely that major developmental mechanisms are conserved, it will be both important and interesting to find out whether the many different types of root apices described in angiosperms (Barlow 1976; Feldman 1984; Abeysekera and McCully 1993; Clowes 1994) utilize similar mechanisms for pattern formation and meristem maintenance.

We thank Frouke Kuyer for excellent art work. C.v.d.B. was sponsored by a grant from the Dutch Organisation of Life Sciences (SLW).

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