THE ACQUISITION OF CELL FATE IN THE *Arabidopsis thaliana* ROOT MERISTEM

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INTRODUCTION

During plant embryogenesis an embryo with cotyledons, a shoot apical meristem, a hypocotyl and a root apical meristem, is formed. The primary root and shoot meristems initiate post-embryonic growth generating all plant organs. The root meristem forms the primary root, and the shoot meristem forms the aerial portion of the plant including secondary meristems. Histological and fate map data have shown that there is no precise correlation between the shoot meristem cells and their descendants (Steeves and Sussex, 1989). This indicates that cell fate is flexible. In contrast, in the root a more strict relationship between differentiated cells and their meristematic ancestors is seen. Little is known about the mechanisms specifying cell fate in meristems.

Here, we focus on the cellular communication that is critical for the formation and functioning of the *Arabidopsis* root meristem. Due to its simple cellular pattern, the *Arabidopsis* root is a suitable system to study cell specification and communication. We have used laser ablations to study the flexibility of cells in the root meristem. Furthermore, we have analysed a number of mutations involved in embryonic as well as secondary and adventitious root formation. Taken together, these results show that root meristem initials learn their fate by positional information and that genes involved in cell specification first act early during embryogenesis.

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EXPERIMENTAL

Laser ablations were performed as described (van den Berg et al., 1995). Living cells could be visualised by the outlining of the cells by the fluorescent dye propidium iodide. Ablation of cells was performed by parking the unfiltered laserbeam (25 mW argon-ion laser, mrc-600, BioRad, Zeiss Axiovert) for 1 sec. on each cell. Successful ablations could be seen by the entering of the propidium iodide into the dead cells (see Fig. 2).

Root mutants were isolated by screening single siliques from 18,000 M1 plants resulting from EMS mutagenesis of dry seeds. Root meristem mutations were selected in the M2 generation. In this way lines were isolated with distortions in root growth or development. Mutants were grouped based on seedling phenotype and subjected to complementation analysis to test allelism.

Histological analysis was performed as described (Dolan et al., 1993; Scheres et al., 1994).

RESULTS AND DISCUSSION

Development of the Root during Embryogenesis

The *Arabidopsis* primary root has a highly regular cellular organisation. It consists of four main tissue types: an inner stele composed of vascular and surrounding pericycle cells, a ground layer of endodermal and cortical parenchyma cells, an outermost layer of epidermal and lateral root cap cells (Fig. 1b, 2a) (Dolan et al., 1993). The basal ends of each cell file are called the initial cells. They are highly constant in number and display regular division patterns. The initial cells divide, generating a new initial and a daughter, which can undergo further divisions and subsequent differentiation. The initials abut four nondividing cells, the quiescent centre, the function of which is unknown.

During the first division of the zygote an apical and a basal cell are generated. The apical cell forms the embryo proper, whereas the hypophysis and the suspensor are generated by the basal cell. The proximal initials are formed from the lowest tier of the embryo proper. The quiescent centre and columella root cap are generated by the hypophyseal cell.

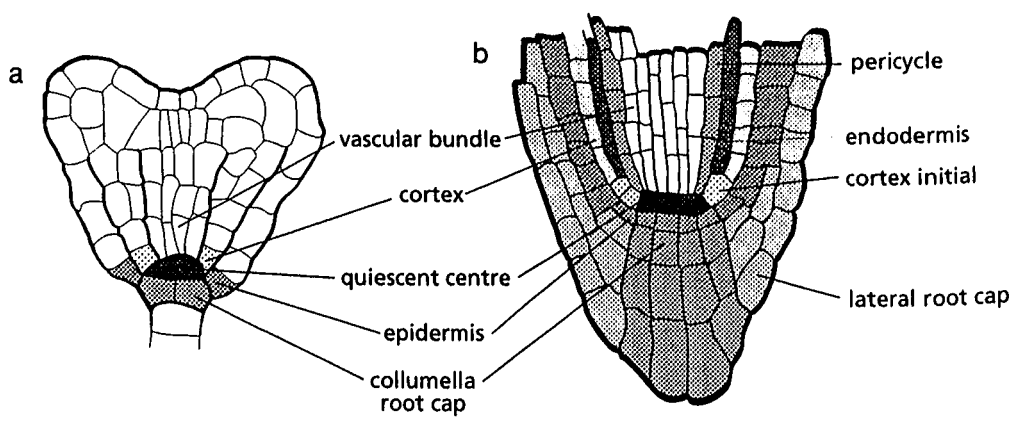

Figure 1. Fate map of the *Arabidopsis thaliana* root; heart stage embryo and seedling.

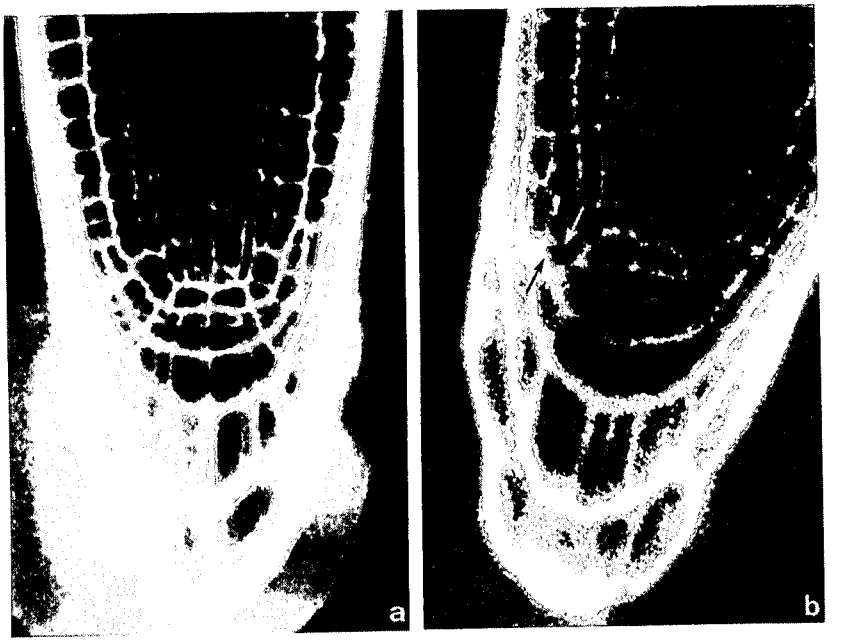

Figure 2. (a) Confocal laserscan of a seedling root stained with propidium iodine. (b) Ablation of an epidermal cell. The dead cell is pressed towards the outside of the root (black arrow) and a cortical cell invades its position (white arrow).

At the heart stage of embryogenesis the cellular organisation of the root apical meristem is completed (Fig. 1) (Scheres et al., 1994).

Communication of Cells in the Root Meristem: The Role of Positional Information

Fate map analysis of cells derived from the shoot apical meristem predicts that cell fate is highly flexible. Positional information rather than clonal origin is thought to regulate cell fate. In contrast, in the root there is a more rigid lineage relationship between meristematic cells and their descendants. This can be taken to suggest that clonal origin makes a contribution to fate determination. To test this in *Arabidopsis* roots, laser ablation can be applied. Cells killed in this way become rapidly flattened and their position is taken up by neighbouring cells (Fig. 2b). The fate of cells in a new position was subsequently studied.

Cell fate decisions in the radial dimension in the *Arabidopsis* root meristem were studied by ablating several proximal initials. After ablation of an epidermal initial, a cortical initial cell invaded (Fig. 2b). This cell formed lateral root cap cells, normally exclusively generated by epidermal initials indicating a switch in fate. Similarly, after ablation of the cortical initial, pericycle-derived cells switched fate; the invading pericycle cell performed the cortex-specific division pattern and generated an endodermis with a casparian strip (van den Berg et al., 1995).

From these experiments, we conclude that the fate of these cells is determined by their position. Furthermore, the signal(s) guiding the functioning of cells is (are) continuously present because switching of cell fates can be observed throughout early seedling development.

What Are the Source and Nature of Positional Information?

To determine the direction of positional signals, we examined further the development of cortical cells. The cortical initial divides first anticlinally generating a cortical daughter. This daughter then undergoes an asymmetric periclinal division forming an inner, smaller endodermal cell and an outer cortical cell (Fig. 3a).

If the cortical initial cell was isolated, by ablating all above daughter cells contacting this initial, the asymmetric division of the isolated cortical cell was prevented (Fig. 3b). This shows that, at least for cortical cells, signals derived from more mature cells of the same tissue type are responsible for fate determination of their initials.

It is clear that positional information acts to reinforce the generated pattern but can lineage still play a role? After all, in the absence of positional information, lineage might determine cell fate. When a single cortical initial was isolated from its daughters, its specification was hindered. This indicates that lineage is of minor importance in cell specification. We concluded that the fate of the proximal initials is instructed by their more mature daughters (Fig. 4a) (van den Berg et al., 1995). Thus, the root meristem initials cannot be seen as the creators of a specific pattern in the plant, whereby the generating cells "know" what to produce. They only conform to an already existing pattern, acting as a copying machine. More apical cells provide the information to copy this pattern.

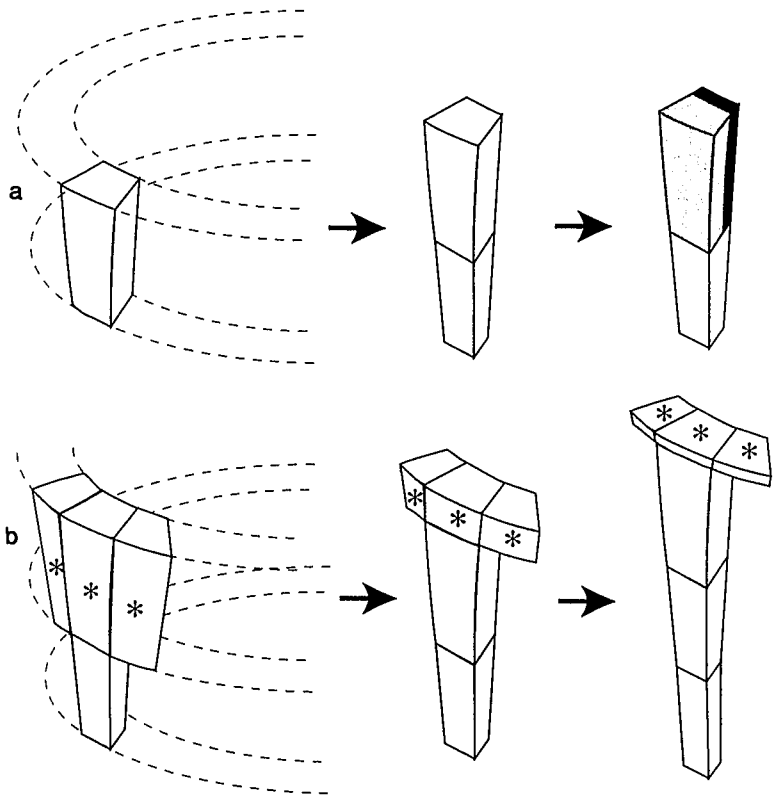

Figure 3. (a) Schematic view of a cortical initial cell. This initial first generates a daughter which performs an asymmetric division forming a cortical (light grey) and an endodermal (dark grey) cell. (b) Schematic representation of ablation of 3 cortical daughters (*) isolating the underlying initial cell. The isolated cell is then unable to perform periclinal divisions, and only anticlinal divisions are seen.

This signal can either act over a long range, providing information along the whole apical-basal axis, or locally in which case a specific group of cells acts as the information source. Ablation of cortical and endodermal cells, 3 cells above the initial, shows that invading pericycle cells still switch fate, indicating that the immediate cortical daughters directly abutting the initials are not the direct source of this positional information.

The most distal cells of the root (quiescent centre and columella) are programmed differently. In these cells, either the signals that determine the radial cell fates are not perceived, or overruling signals are present (Fig. 4b). By ablating the quiescent centre vascular-derived cells move into the former columella position. These cells switched fate and expressed a columella specific marker (a 35S B2-subdomain GUS fusion). In conclusion, the fate of cells along the apical-basal axis is also determined by positional signalling. The source of this signal is currently under investigation. Furthermore, mutants lacking parts of the apical-basal axis are providing more information on how these cell types are established and maintained.

The chemical nature and the method of transport of positional signals are unknown. Cellular components generated in more mature cells could be allocated to the initials via plasmodesmata. It has been demonstrated that at least one transcription factor, *KNOTTED-1*, can be transported from one cell to another (reviewed in Lucas, 1995). KNOTTED-1 is involved in keeping cells in an indeterminate state. Fluorescently labeled injected KNOTTED-1 rapidly moved out of the injected cell into surrounding cells thereby increasing the plasmodesmata size exclusion limit. It is possible that in the root meristem, cell fates are also determined by signals travelling via plasmodesmata. It has been shown that in the root meristem, epidermal cells can transport fluorescent dye into other cells of the epider-

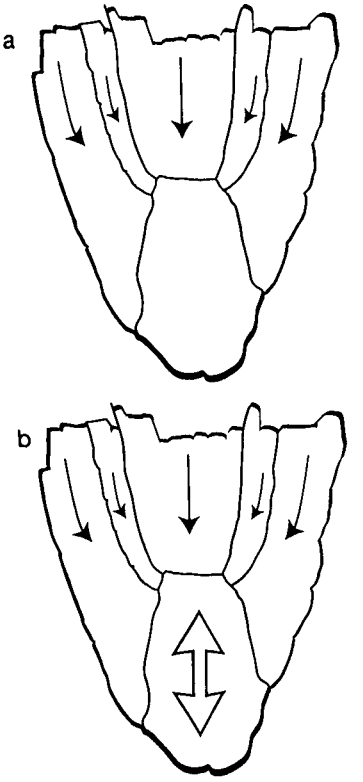

Figure 4. (a) Long range signals determining the fate of cells in the proximal meristem (arrows). (b) Short range positional signals which determine quiescent centre and columella fates (large arrow).

mal cell layer but not into cells in adjacent layers (Duckett et al., 1994). The distribution of plasmodesmata in cell layers could thus be used to restrict the transport of inductive signalling molecules.

Alternatively, laser ablation experiments in the *Fucus* embryo have indicated that the cell wall is involved in cell fate determination (Berger et al., 1994). Contact of one cell with a cell wall of a different cell type causes the cell to switch fate. The basal rhizoid cell in the 2 cell stage embryo was ablated, isolating the thallus cell. Rhizoid cell redifferentiation from the thallus cell was only observed when this cell was contacting the former rhizoid cell wall. If wall-to-wall contact was prevented, isolated thallus cells did not develop a rhizoid. In our ablation experiments the cell wall was not destroyed. This means either that the cell wall is not involved in cell specification, or that the signalling molecules located in the cell wall have a short half life and need to be continuously replenished.

The Function of the Quiescent Centre

The quiescent centre was first identified in maize roots as a group of cells in the centre of the root meristem showing a low rate of cell division (Clowes, 1958). In *Arabidopsis thaliana*, the quiescent centre consists of only four cells. The function of this group of cells has not been demonstrated directly. Barlow (1976) proposed that the quiescent centre cells serve as replacements for initial cells, thereby acting as a stem cell reservoir. Damaging of initial cells should cause the quiescent centre cells to reenter mitosis. It was shown that excision of the root cap in maize roots causes reactivation of quiescent centre cells to reform a complete root cap (Barlow, 1974).

In our experiments, after ablation of cortical or epidermal initials, the dead cells were always replaced by neighbouring initials and not by quiescent centre cells. Thus, apparently, the major function of the quiescent centre is not replacing damaged cells. However, when columella cells were ablated, quiescent centre cells did replace the dead cells. Furthermore, in older roots, quiescent centre cells have been reported to divide occasionally, generating additional cortical cell layers (Rost et al., 1996). Thus quiescent centre cells can replace neighbouring cells, as has been suggested, but no exclusive function for the quiescent centre can be proposed.

A Genetic Approach to Understand Root Development

We used a second, genetic, approach to obtain more information concerning signals involved in root formation and functioning. A number of mutants have been isolated which lack pattern elements in the apical-basal axis of the embryo (Mayer et al., 1991). For example, mutations in the *monopteros* (*mp*) gene interfere with the formation of both the root and hypocotyl during embryogenesis (Berleth and Jürgens, 1993). Cells of the lowest tier of the embryo proper and of the hypophysis display abnormal division patterns from the octant stage onwards. This leads, in the strong mutant phenotype, to a seedling lacking all basal structures.

During wild type embryogenesis, the basal part of the embryo is subdivided into a root and hypocotyl. The root is further subdivided into a prospective embryonic and a meristematic root. We have isolated mutant seedlings which contain an embryonic root but which are directly impaired in the formation of a functional root meristem. Here we will discuss two of them, *hobbit* and *bombadil*. Both show their primary defect in the hypophyseal cell region, the prospective quiescent centre and columella.

Hobbit. Seedlings homozygous for strong *hobbit* (*hbt*) alleles display no root meristem activity at all. They show abnormal root meristem anatomy with regard to both cell shapes and number. Based on anatomy, the quiescent centre and columella root cap region is predominantly affected. We investigated whether these cell types are still present by using cell type specific markers. In wild type plants the more mature cells of the columella contain starch granules. In strong *hbt* mutants these are absent. Moreover, a specific GUS fusion, normally expressed in the root cap, shows no expression in *hbt* seedlings. These results show that the specification of columella cell fate is altered (Fig. 5b). In contrast, a root meristem-specific marker (em101; Topping et al., 1994) is expressed in *hbt* seedlings, showing normal basal cell fate specification (Fig. 5b). We conclude that although the identity of this region is not changed, its cellular specification is lost.

Anatomical analysis of *hbt* embryos shows that the hypophyseal cell undergoes aberrant divisions from early globular stage onwards (Fig. 5b). Furthermore, the periclinal divisions in the epidermal initials that generate the lateral root cap cell are mostly absent. Marker gene expression in *hbt* indeed confirmed that no functional lateral root cap is formed. The *HBT* gene is not only involved in root formation during embryogenesis. Roots generated from homozygous *hbt* seedlings show a similar phenotype and quickly arrest development, showing that *HBT* is involved in all developmental pathways of root formation. *HBT* maps on chromosome 2 and we are currently isolating the corresponding gene.

Bombadil. Besides *HBT*, other genes are involved in root formation including *BOMBADIL* (*BBL*). *BBL* maps on the upper arm of chromosome 3 and we have isolated 2

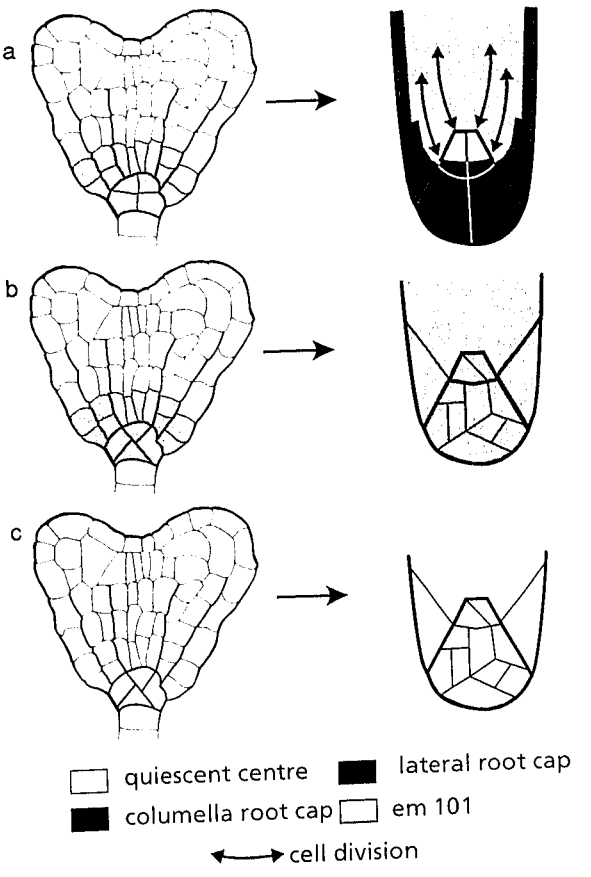

Figure 5. Schematic representations of heart stage embryos and seedling of (a) wild type; (b) *hobbit* and (c) *bombadil*.

alleles to date. Within each allele, the seedling phenotype of *bbl* is variable and two phenotypic classes can be distinguished. The strongest phenotype shows a very short root, a short hypocotyl and small closed cotyledons. The weaker phenotype has a longer root and hypocotyl and larger open cotyledons. The root mostly shows a hook. *bbl* seedlings show a similar phenotype as *hbt* seedlings in that they are also mainly affected in the specification of quiescent centre and columella root cap. *bbl* mutant embryos show more divisions in cells derived from the hypophyseal cell from early stages onwards (Fig. 5c). Later during development, aberrant divisions are seen in other parts of the embryo. In contrast to *hbt*, *bbl* seedlings show no em101 expression (Fig. 5c). This shows that both the regional identity and the cellular specification are lost.

CONCLUSIONS

Based on anatomical data and fate map analysis, the *Arabidopsis* root meristem shows a very rigid cellular organisation. Laser ablation studies show, that cells in the meristem are highly flexible and fate is most likely completely determined by positional information. Furthermore we showed that initial cells learn their fate from more mature cells of the same tissue type. Where exactly this signal is generated is not known. It is likely that all cells in the meristem are able to respond to positional cues. This leads to a model in which a long range signal with an apical to basal direction determines the fates of the different cell layers in the radial dimension (Fig 4a). However, in the most distal part of the root, other cell types are present (quiescent centre and columella). In these cells, fates are also determined by position. We think that these signals act locally, overruling the radial signals (Fig. 4b). We are currently performing laser ablation experiments of cells in this region to determine the direction and source of signals. It will be interesting to see what the fate of the columella and quiescent centre will be if this local signal is removed. It remains to be seen whether the mutants we study lack the same signals. If this is the case, we could conceive that similar positional signals are important for cell fate both during embryogenesis, to pattern the root, and at seedling stage to maintain this pattern. Answering the question whether meristems utilise one or more systems to confer positional information will be a major future challenge.

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