ROOT ANATOMY AND DEVELOPMENT, THE BASIS FOR NEMATODE PARASITISM

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Abstract

Plant parasitic nematodes appear to rely on very specific interactions with root cells to establish a feeding site. To understand these interactions in detail, it is of advantage to achieve a basic understanding of root development. *Arabidopsis thaliana* is a suitable plant to investigate root development genetically and molecularly, and it can act as a host plant for plant parasitic nematodes. The anatomy and the ontogeny of the *Arabidopsis* root can be described in considerable detail. Despite the rigorous lineage relationships in the root, laser ablation experiments demonstrate the presence of continuous information in the root meristem. This information guides cells to differentiate appropriately, according to position. A large spectrum of promoter traps that are specifically expressed in roots are examined in detail, and put into four categories. These expression patterns can be complex, and a relation between the tagged gene and cell type is not always obvious. As a complementary approach, genetic analysis, using specific mutants, is now beginning to unravel key genes that are involved in setting up the pattern of cell differentiation in the root. Combining promoter trap analyses with mutant analysis may create novel strategies for nematode control.

1. Introduction

Controlling an organism by manipulating its favorite food is an ancient strategy. For this control, a thorough knowledge on the organism's gastronomic preferences is required. In this context, this chapter focuses on the obligate partner of parasitic nematodes, the plant. A biological interaction between two different organsims, in this case nematodes and plants, can only be understood in molecular detail if both partners are investigated. For example, study of the *Rhizobium*-legume symbiosis, initially facilitated by the application of bacterial genetics, increasingly relies on analysing the plant response [14]. In a similar vein, knowledge of the genetic program by which the

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root normally develops is a prerequisite for understanding how a nematode selects particular cells and modifies them to serve as a feeding site. Much effort is currently aimed at the establishment of "catalogues" of genes that are up- or down- regulated in feeding sites, using promoter trapping and differential display techniques (see chapters by Fenoll *et al.* and Ohl *et al.*, this volume). Basic knowledge of plant structure and development may help to put this information into developmental perspective. In this chapter we will review recent studies on root development in *Arabidopsis thaliana*, a weed ideally suited for genetic analysis which can be infected by various nematodes [13]. Data on root anatomy and ontogeny, promoter/enhancer trap expression patterns and developmental mutants will be presented. We will conclude with the potential relevance of these data for understanding a fundamental aspect of plant-nematode interactions: the establishment of a feeding site.

2. Anatomy of Arabidopsis Roots

Plant roots have a simple tissue organisation, in which outer rings of epidermal and cortical cells surround a central vascular bundle. *Arabidopsis* roots are a paragon of this regularity. They contain a surprisingly constant number and arrangement of cells in cross-section (Fig. 1A). In longitudinal view, files of each cell type terminate in so-called initial cells (Fig. 1B). A small set of initials for all tissues surround four quiescent cells [6]. This quiescent centre contacts all the initials, an observation that suggests regulatory functions. Quiescent centre and initials together are termed the promeristem, the minimal construction centre of the root [4]. All cells within the promeristem are laid down during embryogenesis, and exhibit the division pattern typical for the root meristem from the heart stage of embryogenesis onward. Cells that leave the meristem as a result of this division pattern progressively differentiate into the various mature cell types as predicted by their position.

3. Ontogeny of Arabidopsis Roots

The ontogeny of an organ describes the way in which it arises from its precursor cells. Ontogenetic studies can be performed by analysing anatomical changes that define a series of developmental stages. This is particularly easy if cells can be readily distinguished either by positional or by morphological criteria. If the latter is not the case, genetic markers can be used to recognise the progeny of single cells ("clonal analysis"). We have performed both anatomical and clonal analysis to study how the primary root meristem is laid down during embryo development. We analysed blue sectors which arose by transposon excision from the *uidA (GUS)* marker gene in transgenic plants. Large sectors mark the progeny of a single embryonic cell. The end points and width of these sectors allowed us to deduce a complete fate map for the *Arabidopsis* root [11]. The root promeristem arises from daughters of both the basal and apical cell which are separated at the first zygotic division: the quiescent centre and

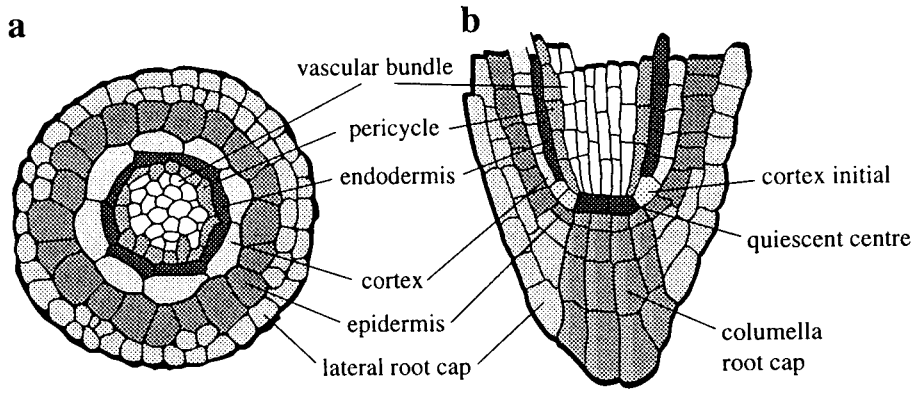

Figure 1. Anatomy of the *Arabidopsis* .root. (A) Transverse view. (B) Longitudinal view.

columella root cap arise from the hypophyseal cell that is, in turn, derived from the basal cell, while the proximal initials arise from the apical cell (Fig. 2). Apparently, the daughters of the hypophyseal cell come to cooperate with the proximal initials to give rise to the functionally integrated root meristem.

The separation of the main tissue types: protoderm, ground tissue and vascular cambium, also occurs early during embryogenesis (cf. protoderm in Fig. 2). These divisions, like the first zygotic division, act as clonal boundaries that separate different cell fates. Root meristem initials that continue to produce protoderm, ground tissue and vascular cells, respectively, are set apart from these tissues at a later stage.

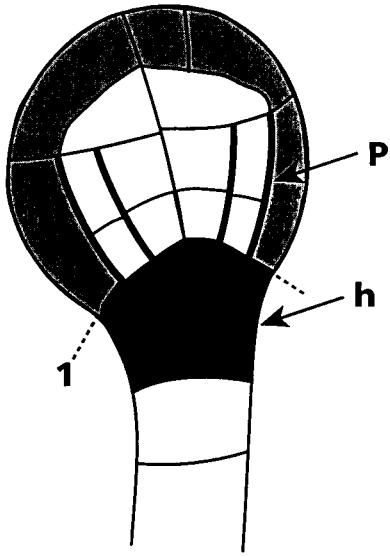

Figure 2. Fate map of early globular embryo. P (light shading): protoderm. h (dark shading): hypophyseal cell. 1: plane of first zygotic division.

4. Flexibility of Cell Fate in Arabidopsis Roots

The strong correlation between cell type and embryonic lineage in the *Arabidopsis* promeristem could be explained in terms of lineage-dependent development after early instructions on cell fate during embryogenesis. On the other hand, positional signalling might continuously determine cell fate. Such signals would have to act at a single-cell resolution, since many layers in the root meristem comprise only one cell. Being superimposed on a rigid cell lineage, such a position-dependency would go undetected. To demonstrate positional signalling in the *Arabidopsis* root, we performed laser ablation experiments. In such experiments, one meristem cell is killed and daughters of neighbouring cells can occupy its position. If the neighbouring cell ends up in a different tissue it crosses a clonal boundary. If commitment of cells were irreversible, the cells would develop into their "old" tissue type. On the other hand, if positional information were continuously operative in the *Arabidopsis* root meristem, the fate of incoming cells after laser ablation would change.

We investigated the ability of root meristem cells to switch fate when they cross the first zygotic division plane (*i.e.* the separation between the quiescent centre and columella on the one hand, and the proximal meristem on the other hand). Upon ablation of quiescent centre cells, the underlying (more distal) columella cells ceased to divide. As a result of this, the dead quiescent centre cells were carried off distally and cell files, continuous with the vascular bundle, were displaced toward the root tip. By using promoter-marker gene fusions, we have shown that these displaced cells switch fate, and display columella-specific gene expression instead of the former vascular- specific expression [15]. Therefore, the boundary set by the first zygotic division does not restrict the developmental potential of the resulting daughter cells.

Upon ablation of cortical and epidermal initial cells, the dead cells are compressed toward the periphery of the root and cells from more internally located tissues take up the position of the ablated cell. Cells derived from the pericycle but now within the cortical cell layer were capable of switching fate and formed both endodermis and cortex. Cortical initials invading the epidermal cell layer formed both epidermis and lateral root cap [15]. We concluded that proximal root meristem cells, despite being clonally restricted to tissue layers at early stages of embryogenesis, are flexible in fate. Hence the early embryonic divisions in the radial plane, like the first zygotic division in the apical-basal plane, are not instrumental in restricting developmental potential. Our experiments are in line with indirect evidence that has been obtained by clonal and chimera analysis of the shoot meristem [*e.g.* 10], and demonstrate that positional information is acting in the regularly patterned root meristem as it is in the shoot.

5. Promoter/Enhancer Trap Expression Patterns in Roots

Patterns of gene expression in an organ form a useful addition to describing organ formation with the aid of morphological criteria. Furthermore, expressed genes that mark a particular cell type or region provide an entrance into the molecular mechanisms that are adopted to specify cellular or regional identity. An easy way to visualise patterns of

gene expression is to transform plants with a promoterless marker gene ("promoter trap"), or with a marker gene containing a minimal promoter that can be activated under the control of nearby enhancer elements ("enhancer trap"). Studies in the fruit fly *Drosophila melanogaster* indicate that the majority of expression patterns found in this way correctly reflect the activity of an endogenous gene that is located nearby the marker gene insertion [*e.g.* 16]. A convenient marker gene in plants is the ß-glucuronidase (*uidA*) gene [8], which converts a colourless substrate into a blue precipitate. A joint promoter trap screen to identify nematode-controlled genes, performed as an EC-AIR concerted action, is described elsewhere in this book (see chapters by Ohl *et al.* and Fenoll *et al.*, this volume). We have investigated in some detail lines with root-specific expression patterns. In addition to these lines, we have analysed other root-expressing promoter/enhancer traps. Below we will describe a variety of expression patterns that we have observed in *Arabidopsis* roots, which can be classified broadly into four different correlation groups: tissue type; cell type; differentiation stage-dependent; complex (Table 1).

5.1. TISSUE TYPE

The protoderm, ground tissue, and provascular tissue form the three major tissue types in vascular plants. In the root, the distal root cap can be envisaged as a fourth major tissue. The *AX92* marker was identified as a ground-tissue marker in root and hypocotyl [5], and this has been confirmed in *Arabidopsis* (Claudia van den Berg; Jocelyn Malamy, pers. comm.). In addition, we have identified marker gene expression patterns in roots that coincide with the vascular tissue (pMOG553-643) and root cap (CaMV 35S::B2 subdomain). We have not yet identified lines which stain all of the root epidermis specifically.

5.2. CELL TYPE

Within each of the major plant tissues, several distinct cell types can be identified by morphological criteria. Within the root, these are from periphery to center: the root hair-carrying epidermis (trichoblast); the non-hair epidermis (atrichoblast), the cortical parenchyma; the endodermis; the pericycle; phloem and xylem vascular elements; companion cells; vascular parenchyma and root cap cells. Recently it has been shown that the *GL2* gene is expressed in non-hair epidermal cells [9]. In our analysis, we have investigated a number of cell-type specific markers. Cortex-specific, endodermis-specific as well as pericycle-specific expression patterns have been detected (Table 1). We have focused on specific markers within the root cap region. The root cap presents a complex case, since the functional cap cells slough off the root and they may undergo programmed cell death at their final stage of development (Fig.1). It is therefore possible to define the "terminally differentiated root cap cell" in at least two ways: i) the fully functional cells still attached to the root, *e.g.* the columella cells with the accumulated starch grains that are thought to be involved in the gravitropic response. ii) the cell that is lysed and detached from the root. We have detected marker genes that are expressed specifically in either of these two cell types, as well as markers specific for the lateral root cap (Tab. 1).

TABLE 1. Characteristics of marker lines. All three- and four-digit markers are from the Concerted Action screen using the pMOG533 insertion. Sources of other markers are indicated in the text.

Class	Marker line	Characteristics
Tissue-specific	643	vascular bundle
	CAMV 35S::B2	root cap
	PKU 6	root cap
	ET 271	root cap
Cell-type-specific	PKU 14	columella
	ET 283	columella
	ET244	lateral root cap
Differentiation-phase-specific	124	quiescent centre + vascular initials?
	300	columella tiers
	463	columella-tiers
	648	columella-tiers
	359	columella-tiers + lateral root cap
	649	columella-tiers + lateral root cap
	915	columella tiers + lateral root cap
	1066	columella tiers + lateral root cap
	438	meristematic zone
	826	meristematic zone
	654	elongation zone
Complex	*POLARIS*	root tip
	516	root tip
	174	columella tiers, part of embryonic hypocotyl
	134	columella tiers + vascular tissue
	959	columella tiers + vascular tissue
	1027	columella + vascular tissue
	1004	root cap + vascular tissue

5.3. DIFFERENTIATION STAGE-DEPENDENT

These markers define zones which can be grouped by common functional characteristics, which are shared by several cell types or tissues. Examples are marker genes that specify collectively all initial cells, the meristematic zone, and the elongation zone. As discussed above, markers that are expressed in specific layers of the root cap can also fall into this class, depending on the definition of the terminal root cap cell type.

It must be noted that caution must be taken when an expression pattern seems to correlate completely with a cell type. For example, the pMOG553-1027 marker is specific for the columella root cap at the seedling stage, but is procambium-specific in the embryo. When this marker is expressed in a developmental mutant, it cannot be concluded that the expressing cell is either one of these two cell types.

5.4. COMPLEX EMBRYONIC OR POST-EMBRYONIC EXPRESSION

Complex markers define zones in the embryo that cannot (yet) be grouped by common functional or cell type characteristics. For example, three marker lines show GUS expression in a small region of the root cap, but also in vascular cells immediately proximal to the quiescent centre. It remains to be established whether these gene expression patterns convey that the two cell types have something in common, or, alternatively, whether the marker correlates with postional values that are set up *e.g.* by the quiescent centre. A marker for which it is more clear that it substantiates a positional value, is the *em101 (POLARIS)* gene, which is expressed in the distal root tip in a constant region from heart stage embryogenesis onward.

6. Mutational Analysis of Root Development

The *Arabidopsis* root develops by a stereotyped scheme of cell divisions that starts during embryogenesis, superimposed on a continuously present system of signals that determine cell fate. Identification of genes that are involved in generating, perceiving, and responding to the signals that allocate cells to tissues in the root is a major strategy to unravel the molecular details of this process. Below we will summarize the results of genetic screens aimed at identifying genes that are required for the formation of specific elements of the cellular pattern that comprises the root.

6.1. RADIAL ORGANISATION

A number of mutants have been described that alter the radial organisation of the root [1; 12]. Given the allele frequencies of the identified loci, probably most of these genes are as yet unidentified. The three mutants *shortroot, scarecrow* and *pinocchio* are affected in the specification of cortex and endodermis from the ground tissue. *scarecrow* and *pinocchio* have recently been shown to represent two different mutations in the same gene (P.N.Benfey, pers. comm.). *wooden leg* and *gollum* interfere with the specification of the vascular tissue. Noteworthy, the layer-specific phenotypes persist in the hypocotyl, and all five mutants have an embryonic phenotype throughout the embryonic axis. Hence all genes identified so far that influence the pattern affect the complete seedling axis from embryogenesis onward. The tentative conclusion is that the information in the root meristem originates as the result of gene activities during embryonic radial pattern formation. Based on these results and those from laser ablation experiments, it becomes attractive to envisage the root meristem as a group of dividing cells that is competent to react to signals from more mature cells. These signals, in turn, depend on the correct activity of pattern formation genes that are first active during embryogenesis. Since all the radial mutants display similar phenotypes in lateral roots and in roots derived from callus, the corresponding gene activities are not restricted to embryogenesis, but appear to be employed again when secondary roots are formed.

Cells in the distal region of the root, containing the quiescent centre and the root cap, are programmed differently since they do not form elements of the radial tissue

pattern. Yet the laser ablation experiments show that this region also is programmed by positional information. In the next section we will present genetic data that suggest the great importance of this distal region in establishing a root meristem.

6.2. MERISTEM SPECIFICATION

In addition to radial specification of cell layers, the root and root meristem are specified as elements of the apical-basal embryonic pattern. Clonal analyses showed that the boundary between the root and the hypocotyl does correlate, but not with cellular precision, to early embryonic divisions [11]. This indicates that, during early pattern formation, root and hypocotyl fate are connected. A few *Arabidopsis* genes have been described which are required for the formation of a root. Among these, the *MONOPTEROS* gen [2] has been analysed in detail. This gene appears to be required for the specification of both root and hypocotyl in the embryonic context. The *mp* phenotype also indicates the intimate relation between root and hypocotyl specification.

Mutations in the *ROOT MERISTEMLESS* loci display no embryonic phenotype but lack post-embryonic cell division in the root meristem (3; Viola Willemsen and Ben Scheres, unpublished). These *rml* mutants appear to be disturbed in the re-initiation of cell division within the root meristem upon germination.

We have concentrated on defining loci which are involved in the programming of a correctly patterned root meristem. The fate map of the *Arabidopsis* seedling shows that the majority of the primary root cells arise from the root meristem. However, cells within a small region covered with root hairs that connects root and hypocotyl originate from a different region in the embryo and are referred to as the "embryonic root". We performed a genetic screen for mutant seedlings that contained the embryonic root, but lacked an organised promeristem. In this chapter we will discuss in some detail four genetic loci that upon mutation confer embryonic defects in the hypophyseal cell region (*e.g.* the prospective quiescent centre and columella):*HOBBIT, BOMBADIL, ORC,* and *GREMLIN* (Figures. 3,4). A number of other mutants were identified, which have similar phenotypes to the ones stated above but which are not allelic. All the mutants are fully recessive and, with the exception of *orc*, seedling-lethal or sterile.

6.3. THE HOBBIT PHENOTYPE GROUP

The *HOBBIT* locus is defined by a series of independent allelic mutations which lead to a very similar, "root meristemless" appearance (Fig. 4; Willemsen *et al.*; in prep.). Seven independent alleles were identified in our screen, and four more alleles were kindly provided by Prof. G. Jürgens (Univ. Tübiningen, Germany) and Drs. H. Höfte and C. Bellini (INRA Versailles, France). Seedlings homozygous for strong *hbt* alleles display no root meristem activity, while seedlings homozygous for weak alleles allow some residual activity. All seedlings homozygous for *hbt* alleles have abnormal root meristem anatomy. The most conspicuous anatomical defects are the irregularities in cell shape, number, and arrangement of the columella and quiescent centre region. Mutants homozygous for strong *hbt* alleles contain no differentiated columella root cap, based on the absence of starch granules. *hbt* mutants carrying either strong or weak alleles show

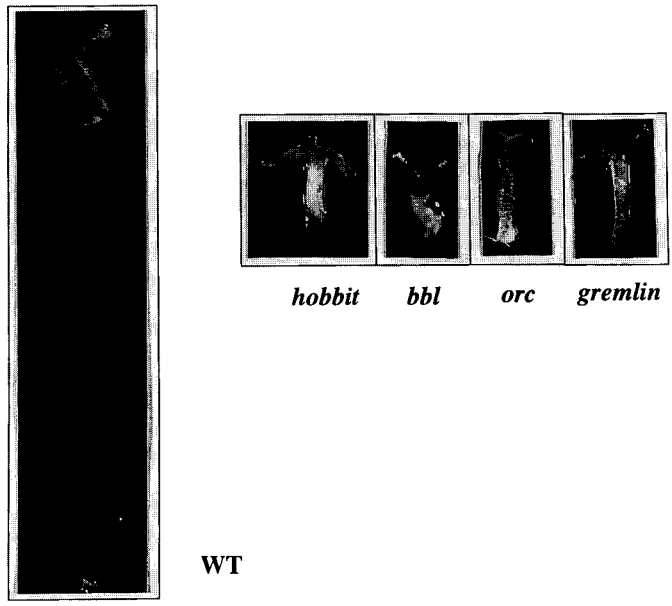

Figure 3. Seedling appearance of wildtype and "hypophyseal cell group" mutants.

abnormalities in division pattern within the hypophyseal cell from early globular stage embryo onward. These abbreviations seem restricted to the hypophyseal cell at early stages of embryogenesis, but the proximal initials, most notably the epidermal initials that should form a lateral root cap, can also become abnormal (Willemsen *et al.;* in prep). This mutant phenotype suggests three functions of the *HBT* gene: i) specifying hypophyseal cell descendants; ii) triggering of activity in the proximal meristem, and iii) proper formation of a lateral root cap. It remains to be clarified whether the second and third function are a direct downstream result of the presence of a correctly programmed hypophyseal cell.

The root phenotype in *hbt* mutants is not embryo-specific. Adventitious roots from *hbt* mutants, generated from the hypocotyl of seedlings or via tissue culture, have the characteristic mutant phenotype and arrest development. Therefore the *HBT* gene, unlike the *MP* gene, is not required just for embryonic root formation but for root formation in all developmental contexts [2; Willemsen *et al.*, in prep.]. However, only the root meristem forms abnormally. *Hobbit* seedlings contain basal root hairs and express the basal embryo marker gene *POLARIS* .

6.4. THE BOMBADIL PHENOTYPE GROUP

Further evidence for more than one gene acting in a similar specification pathway comes from the phenotype of the *bbl* mutants. *bbl* seedlings are distinguishable from *hbt* seedlings (Fig. 3), and they form a different complementation group with different map

MATURE EMBRYO STAGE

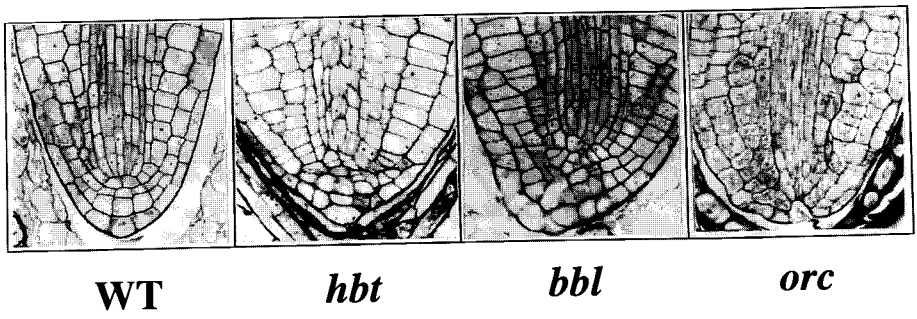

Figure 4. Anatomy of wildtype and "hypophyseal cell group" mutant mature embryos

position than *hbt*. While the seedling phenotypes are distinct, the anatomical deviations in globular and heart stage *bbl* mutant embryos are similar to those observed in *hbt* mutant embryos. At the mature embryo stage, the shape of *bbl* root poles differs. Marker lines indicate that the cells in the position of the wildtype columella do not acquire root cap characteristics in strong *bbl* mutants. Moreover, *POLARIS* gene expression is absent in a strong *bbl* mutant. As with the *hbt* phenotype class, a number of non-allelic mutations exist which give rise to seedlings closely resembling *bbl* seedlings. These are currently subjected to mapping and pairwise complementation analysis, and analysis of the embryonic phenotype.

6.5. THE ORC PHENOTYPE GROUP

Mutants of the *orc* phenotype group are characterized by the presence of a reduced root (Fig. 3). For none of these mutants is an allelic series available yet, which hampers somewhat the analysis of the phenotype. Under the assumption that the mutants represent full loss-of-function, the *ORC* and *GREMLIN* genes, which interfere with the formation of the hypophyseal cell descendents at least from the heart stage onward, do not completely block meristematic potential: limited meristem activity is observed. The embryonic phenotype is always conspicuous in the hypophyseal cell region, but includes variable anatomical features. In *orc* embryos and seedlings, sometimes the cell in place of the wildtype hypophyseal cell appears not to have divided at all (Fig. 4). In other cases, randomised divisions in the *orc* hypophyseal cell region result in a cluster of small cells at the position of the hypophyseal cell decendants. The latter anatomical feature is also characteristic for *gremlin* seedlings.

The observed variability in division patterns interferes with simple classification of the *orc* group of mutants. The use of promoter/enhancer trap lines will be essential to understand fully the nature of the defect in these mutants. Mutants specific to hypophyseal cell programming would be expected to have altered tissue-specific marker

gene expression in this but not other regions of the embryo/seedling. Alternatively, some of the *orc* group mutants may be explained by a higher sensitivity of the hypophyseal cell to mutations in the machinery for cell division. The extreme regularity of hypophyseal cell divisions in comparison with the embryo proper may put more stringent requirements on the division apparatus within these cells during embryogenesis. For example, these mutations may have impaired, but not eliminated, the activity of cytoskeletal proteins or their associated motor proteins, which are involved in specific asymmetric cell division and cell fate segregation.

7. Conclusions

Normal root development is relevant for understanding plant-nematode interactions in two ways. Firstly, recognition and feeding site selection seem connected to developmental cues, because the nematode is able to find its way in the root tip. Secondly, the cellular modifications that accompany the establishment of a feeding site are performed within a given developmental context: only particular cells are chosen to serve as a feeding site. Below we briefly comment on the significance of our results for these two aspects of the interaction.

The Arabidopsis root has a simple structure which allows descriptions of plant-nematode interactions with cellular precision [7]. From the penetration behaviour and feeding site selection of cyst and root-knot nematodes, it appears that nematodes can recognise cell types and differentiation status (see chapters by von Mende; Golinowski *et al.*; and Bleve-Zacheo and Melillo, this volume). One possibility is that nematodes sense cell-specific molecules. Alternatively, nematodes could read the positional information that instructs root cell differentiation directly. In either case, we should like to know the identity of the relevant plant molecules. Understanding how molecules instruct cells to be different at the relevant positions in the root is expected to follow from further studies on the pattern formation genes currently under analysis. These molecules, or altered versions of them, may become future tools to mislead the parasitic nematode in its search for the proper feeding site.

Insights into the mechanisms of cell fate determination is relevant to understand how nematodes change particular cells into highly specialised feeding structures. Establishment of a feeding structure will involve plant cell division as well as cell differentiation pathways. Feeding cell-upregulated genes are being catalogued currently. From the large-scale screening of promoter trap lines it has become obvious that a relatively high proportion of up-regulated genes is induced in lateral roots (see Fenoll *et al.*, this volume). Whether such genes represent genuine root-specific functions that are adapted by the nematode, or whether the majority of genes is aspecifically derepressed in lateral root primordia, remains to be seen. Genetic analysis of cell determination in the root, as described above, is expected to provide key regulator genes that induce the many differences in gene expression in the various root cells and thereby can aid in the effort to understand the changes that nematodes induce. We have shown that promoter traps can display a variety of expression patterns of which it is not always easy to determine whether they represent cell differentiation events. Studying promoter trap expression

patterns in the root mutants will identify the promoter trap lines that are correlated to cell differentiation. Careful analysis of such selected tags during nematode infection may provide valuable information on cellular differentiation characteristics that are essential for feeding cell induction. Furthermore, understanding how cell differentiation pathways are connected with each other seems pivotal if one wants to achieve effective nematode control using plant genes without unwanted secondary effects on plant development. Cell cycle regulation is also likely to be of importance for feeding site establishment, as judged from the expression pattern of *CDC2* and cyclin genes (see Gheysen *et al.*, this volume). In this case also, the combination of cell cycle marker genes with genetic approaches can be useful. It will for example be interesting to determine whether parasitic nematodes are capable of regulating the *RML* loci, which have been shown to be involved in the stimulation of cell division in the root.

Whether any of the expectations stated above will turn out to have practical value remains to be seen, but it is becoming clear that continuing studies on root development will further enhance our knowledge of the dinner table preferences of parasitic nematodes. We hope that such knowledge will enable the birth of novel concepts in pest control.

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