

## Genetic analysis of root development

John W. Schiefelbein, Susan K. Ford, and Moira E. Galway

*The development of roots represents a useful system to study many aspects of plant morphogenesis. Roots display a relatively simple growth pattern and possess radial symmetry in their cell layers. These features aid studies of pattern formation, meristem activity, and cell differentiation. Recent genetic approaches to the study of root development have focused on *Arabidopsis thaliana*. This research has led to the identification of several genes that regulate morphogenesis in roots. Genes have also been identified that are involved in the ability of roots to respond to various external stimuli, including gravity and obstacles.*

**Key words:** root development / *Arabidopsis thaliana* / root hairs / cell development / pattern formation

THE DEVELOPMENT of plant organs requires the formation of specific patterns of cells and the differentiation of those cells into a mature form. Roots represent a useful organ for studying pattern formation and cell differentiation in plants because: (1) the root meristem is accessible and not enclosed by developing organs or primordia; (2) the root is free of pigments and therefore essentially transparent; (3) there are relatively few differentiated cell types in roots; and (4) root morphogenesis in many plants occurs in a continuous and relatively uniform fashion without any significant developmental transitions. Root development is also influenced by a variety of external stimuli, such as gravity and temperature. This enables roots to be used to study the effects of these stimuli on organ development.

Genetic analysis of root development has traditionally not received a great deal of attention, in part because roots are subterranean organs. This property has often inhibited attempts to identify variants defective in root development. Research in the developmental genetics of roots has recently benefited from the use of *Arabidopsis thaliana* as an experimental organism. This review summarizes our

current understanding of the genetics of root development with an emphasis on examples from *Arabidopsis*. Two basic categories of root developmental mutants are described: morphological mutants that affect pattern formation or cell differentiation, and mutants that are unable to respond in a normal manner to external stimuli.

### Development of roots

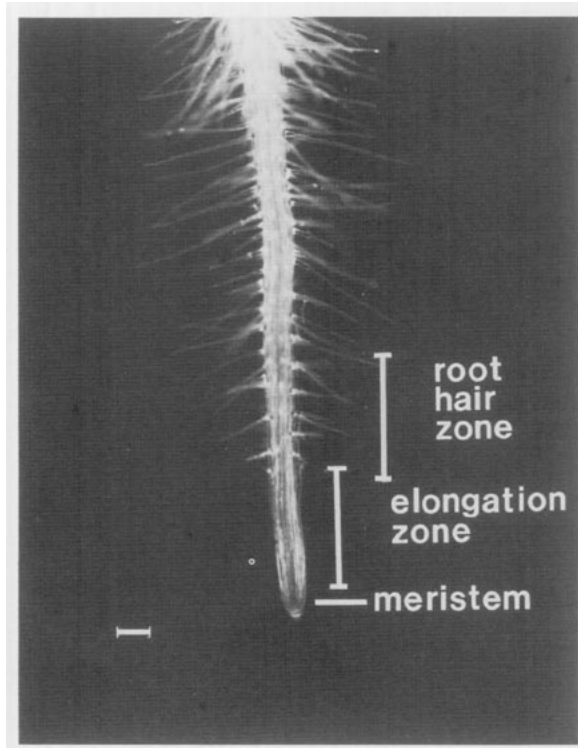
Although the size of individual roots and the pattern of branching in root systems vary widely, the basic developmental features of roots appear to be similar in all vascular plants. Most primary roots (tap roots) grow continuously by means of cell divisions and cell expansions that occur at or near the root apex. These processes lead to the formation of files of differentiated cells.<sup>1</sup> Because root development occurs in a continuous and relatively uniform fashion, all stages of root morphogenesis are present in the growing root. The spatial separation of developmental processes is one of the advantages of studying roots, for it allows one to examine cells at all developmental stages in a single organ at any one time.

Stages in root development can be conveniently divided into 'zones' along the primary (apical-basal) axis of the root (Figure 1). The root meristem, formed during development of the plant embryo, is a region of continuously-dividing cells that produces the basic cell types and defines their organization. A detailed description of the root meristem, including the precise organization of meristematic stem cells and the fates of cells that emerge from the meristem (root cell initials), has been achieved in the water fern *Azolla*. The precise placement and timing of each division in the unicellular meristem of *Azolla* roots has been determined, resulting in a complete cellular fate map.<sup>2</sup> An understanding of the root meristem of higher plants has been complicated by several factors: (1) the root meristem is multicellular; (2) it generally displays an indeterminate growth pattern; and (3) it includes a unique set of cells, the quiescent center, which undergoes relatively infrequent cell divisions.<sup>3,4</sup>

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**Figure 1.** *Arabidopsis thaliana* root with zones of development indicated. Seedling was grown on a vertically-oriented Petri plate under continuous light for 4 days. Bar = 100  $\mu\text{m}$ .

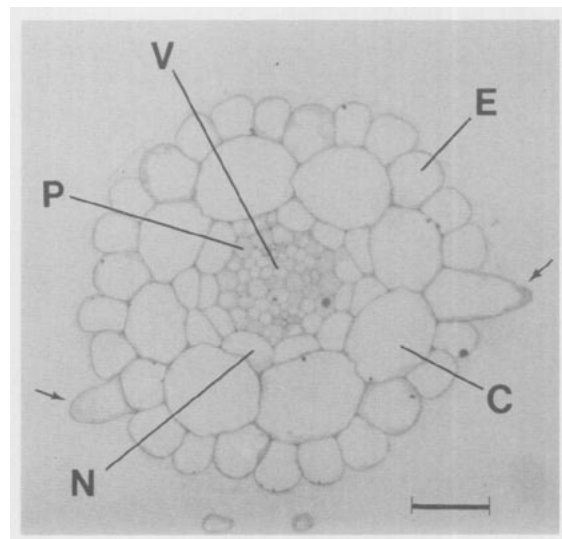
The elongation zone of the root includes small, densely-cytoplasmic cells that are primarily expanding in size. Controlled cell expansion in this region leads to the characteristic elongated cells of roots and provides the force that extends the root meristem into the soil. Differential cell elongation across this region of the root leads to changes in direction of root growth. The specialization zone (often referred to as the root hair zone) contains cells that are differentiating into their final form and function. The most conspicuous cell type in this region is the root hair cell (Figure 1). It should be noted that the differentiation of some root cells (e.g. root cap cells) does not occur in this region. Also, the developmental zones of roots are not sharply separated; there is overlap in the cellular processes occurring in various zones. Branching of roots occurs at some distance from the root apex through the formation of lateral roots. Lateral roots develop from differentiated cells in a special layer, the pericycle, that redifferentiate to elaborate a root that forces its way through the overlying cell layers and into the external environment.<sup>5</sup>

In addition to an apical-basal polarity, roots also possess radially-organized layers of cells. This is apparent when transverse sections of roots are examined. Moving outward from the vascular cylinder, which is located in the center of the root, the layers include the pericycle, endodermis, cortex, and epidermis. In *Arabidopsis*, this organization is particularly simple, because each layer is one-cell thick (Figure 2). The total number of cells in each layer is also relatively invariant for a particular segment of the root.<sup>6</sup>

### Root developmental mutants

Although morphological studies have led to an understanding of some of the broad outlines of root development, relatively little is known about the molecular mechanisms that control root morphogenesis or the ability of roots to respond to external stimuli. Root developmental mutants are useful in this regard because they define specific genes controlling root development. Mutants also serve as a starting point for gene isolation and subsequent molecular analysis.

Root mutants have been isolated from a variety of plant species (see reviews in refs 6-29), but *Arabidopsis* has in recent years been used increasingly



**Figure 2.** Transverse section of *Arabidopsis* root (ecotype Columbia). The cell layers are epidermis (E), cortex (C), endodermis (N), and pericycle (P) around the central vascular cylinder (V). Note the portions of root hairs projecting from some of the epidermal cells (arrows). Bar = 20  $\mu\text{m}$ .

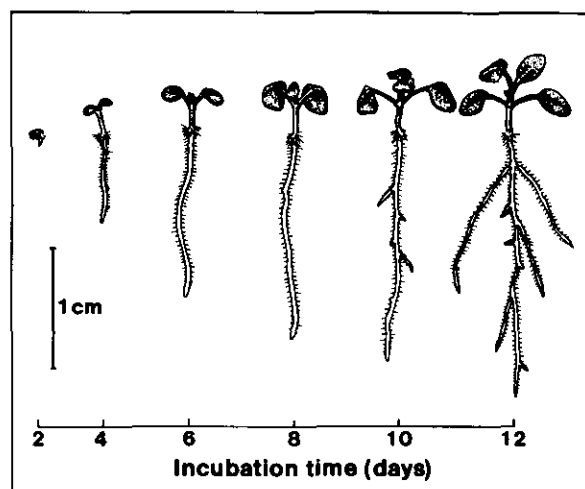
for genetic analyses of root development. The small size of *Arabidopsis* facilitates genetic analyses, as seedlings can be grown in large numbers on Petri dishes, thereby making their root systems readily accessible to examination. As an example, more than 10,000 *Arabidopsis* seedlings can be grown in one cubic foot of space. Following analysis in Petri dishes, individual seedlings of interest can be transplanted to soil to produce seed. The growth of *Arabidopsis* seedlings in Petri dishes is shown in schematic form in Figure 3. The ease with which large numbers of *Arabidopsis* plants can be studied has recently led to the isolation of many root developmental mutants following chemical, radiation, and T-DNA mutagenesis experiments (refs 10-14; J. Schiefelbein, unpublished results). In the following sections, we focus on several sets of mutants that serve to illustrate the value of genetic approaches to root development.

### Morphogenesis mutants

#### Root pattern formation

One of the earliest events in plant embryonic development is the establishment of the apical-basal axis of polarity. This results in the formation of the four basic regions of the seedling, which are from top to bottom, the shoot meristem, cotyledons, hypocotyl, and root. Several mutants of *Arabidopsis* have been identified that appear to affect the formation of the basal (root-producing) portion of the embryo.<sup>13</sup> Mutant alleles of one gene (*monopteros*) result in seedlings that completely lack root and hypocotyl regions, possessing only cotyledons and the shoot meristem. Mutations in a second gene (*gnom*) delete the root and alter the cotyledons, indicating that this gene influences the development of two non-adjacent regions of the apical-basal axis. These results demonstrate a role for both the *monopteros* and *gnom* products, acting in either a combinatorial or hierarchical fashion, in establishing the basal pole of the embryo.<sup>13</sup> Additional insights into early root development and the establishment of the root meristem should be obtained by the analysis of the *monopteros/gnom* double mutant and by the characterization of other embryo-specific mutations with defects in root development.<sup>14,15</sup>

Another level of pattern formation in roots is the establishment of the radial pattern of cell layers (Figure 2). Genes involved in this process in the *Arabidopsis* root have recently been discovered (P.N. Benfey *et al*, submitted). Mutations have



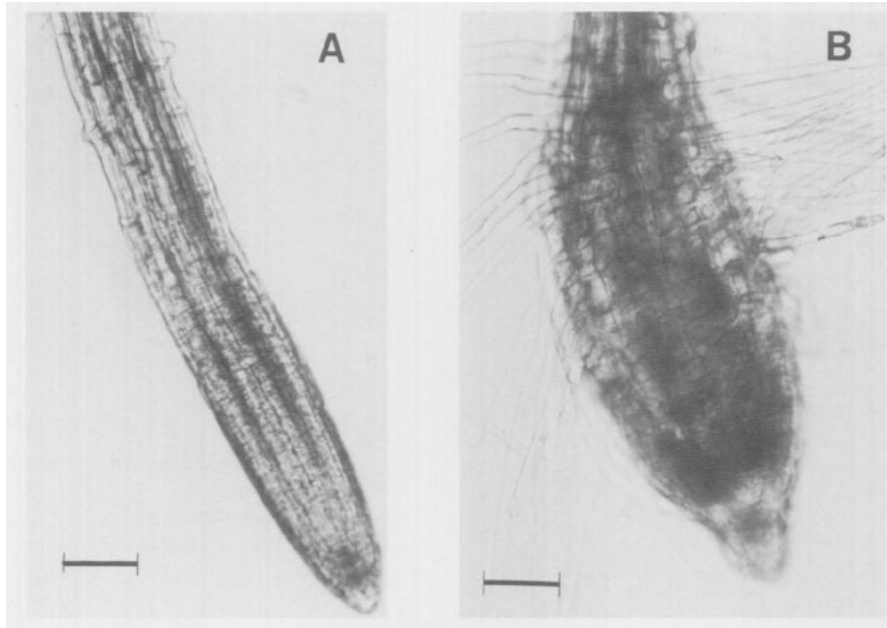
**Figure 3.** Schematic drawing of a developmental profile derived from *Arabidopsis* seedlings axenically grown on agarose in sealed Petri plates for various lengths of time.

been identified that affect specific cell layers; either deleting an entire layer of cells, or altering the size, shape, or number of cells in a particular layer (Figure 4). In addition, mutations have been found that appear to alter the activity of the root meristem and thereby disrupt morphogenesis of the entire root (P.N. Benfey *et al*, submitted). The characterization of mutants in this group should lead to an understanding of the mechanisms that serve to organize and regulate the root meristem, including the timing of cell divisions, the establishment of the lanes of cell division, and the establishment of specific cell layers.

Another type of patterning in roots can be found within individual cell layers. For example, the epidermal layer consists of two distinct cell types: cells that form root hairs (long, cylindrical projections from individual epidermal cells) and cells that do not. In *Arabidopsis*, a relatively predictable pattern of root-hair cells and non-hair-forming cells has been detected in the epidermal layer. Mutants have recently been identified that alter this pattern by producing different numbers of root-hair cells versus non-hair cells; the affected genes may therefore act to establish or maintain the normal epidermal cell pattern (M. Galway, J. Schiefelbein, unpublished results).

#### Root cell differentiation

The differentiation of a specific type of root cell has been explored through the characterization of root hair mutants in *Arabidopsis*.<sup>11</sup> Root hair development involves localized changes in the shape of particular epidermal cells. Plant cell shape is determined by the



**Figure 4.** Comparison of roots from wild-type (A) and mutant (B) *Arabidopsis* plants. The mutant plant possesses a defect that causes abnormal cell expansion in a single cell layer. Magnification = 100X. Bar = 100  $\mu$ m.

shape of the cell wall; root hair development therefore represents an opportunity to study the role of localized loosening and synthesis of the cell wall in the generation of cell shape. Furthermore, because not all epidermal cells normally differentiate into root hairs, root hair development also provides a chance to investigate the ability of specific epidermal cells to differentiate themselves from other cells within the same layer.

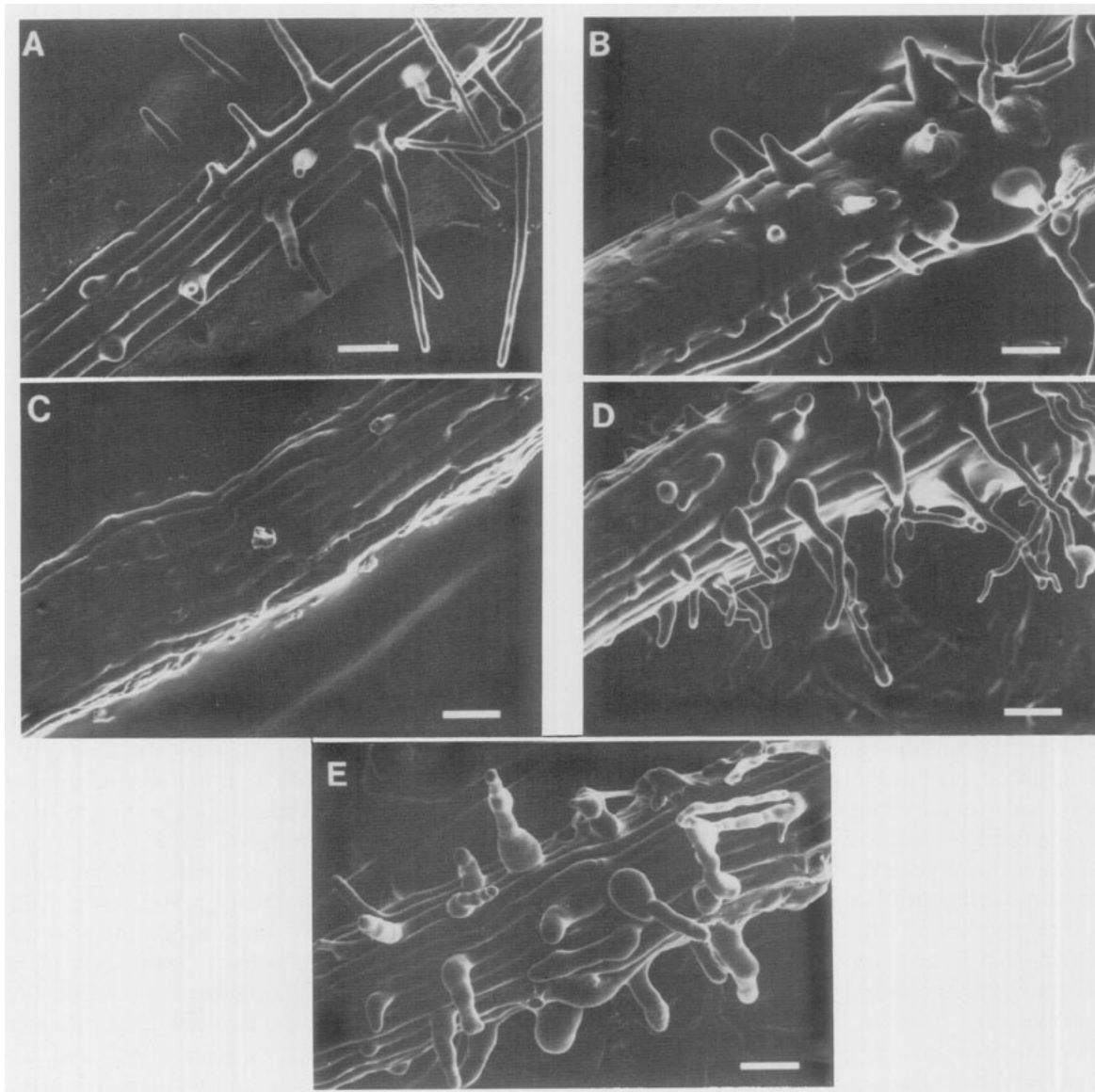
Genetic analysis of root hair development is facilitated by the fact that root hairs are dispensable; plants lacking root hairs are viable and able to reproduce normally.<sup>11</sup> To date, four genes have been described that are required for normal root hair development in *Arabidopsis*.<sup>11</sup> One of these genes (*RHD1*) appears to encode a product involved in an early step in the formation of the root hair. *RHD1* mutants form an abnormally large bulbous region on the epidermal cell wall during the initial phase of hair formation, perhaps because of a defect in localized cell expansion (Figure 5). The *RHD1* gene may be one of a group of genes that is expressed in epidermal cell precursors and is required for the initiation of root hairs.

Three *Arabidopsis* genes have been described that are required during a later phase of root hair development known as root hair elongation. During this phase, root hairs expand in size by localized growth at their tip. This represents a type of polarized growth that is also found in pollen tubes,

fungal hyphae, and other tubular-shaped cells.<sup>16,17</sup> Mutations in the *Arabidopsis RHD2*, *RHD3*, and *RHD4* genes all lead to root hairs with an abnormal morphology (Figure 5). Mutations in the *RHD2* gene prevents hair elongation entirely and leads to a 'stubby' hair phenotype. Defects in the *RHD3* and *RHD4* genes alter tip growth to produce hairs that are 'singed' or 'bulging' in appearance, respectively. Although the products encoded by these *RHD* genes have not been identified, they are likely to affect cellular factors that normally control root hair development, such as actin cytoskeletal function or  $Ca^{2+}$  fluxes.<sup>18,19</sup>

Three of the four *RHD* genes (*RHD1*, *RHD2*, and *RHD4*) appear to alter only root hair development because mutations in these genes do not cause a detectable change in other plant characteristics. However, *rh3* mutants display aberrant cell expansion in many plant tissues. For example, the roots of *rh3* mutants are approximately 70% as long as wild-type, due to a reduction in cell elongation.<sup>11</sup> The *RHD3* gene product is therefore likely to be involved in a general aspect of plant cell expansion.

Based on the analysis of *rh* double mutants, a developmental sequence of gene activity can be outlined (Figure 6; ref 11). The *RHD1* gene is positioned at the earliest stage (initiation) because its mutant phenotype is detected in all double mutant combinations. The *RHD2* gene is next in this sequence, because *rh2* mutations prevent hair



**Figure 5.** Scanning electron micrographs of developing *Arabidopsis* wild-type and mutant root hairs in the root hair zone. Each micrograph is oriented such that the direction of root growth is toward the lower left of each panel. (A) wild-type, (B) *rhd1*, (C) *rhd2*, (D) *rhd3*, (E) *rhd4*. Bar = 50  $\mu\text{m}$ .

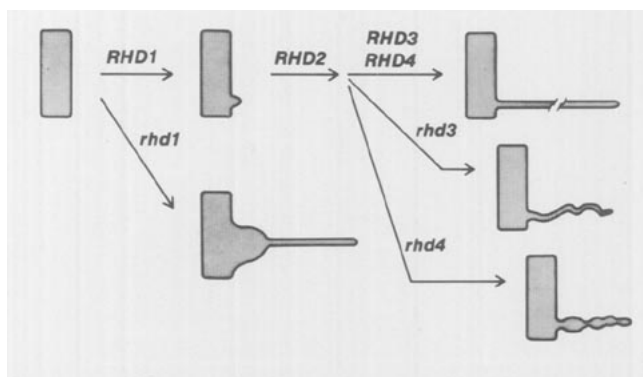
elongation and are epistatic to *rhd3* and *rhd4* mutations. The *RHD3* and *RHD4* genes are both required during the process of hair elongation, and probably act in separate pathways because the *rhd3/rhd4* double mutant reveals an additive interaction between these two mutations.<sup>11</sup>

#### *Stimulus perception/response mutants*

The development of roots is influenced by a variety of external stimuli such as gravity, temperature,

moisture, aeration, and physical obstacles. These stimuli can alter the direction or degree of cell expansion, the amount of root branching, or the structure of root cells (see reviews in refs 20, 21). Several genetic studies have focused on understanding how roots are able to perceive and respond to these external stimuli.

Roots normally display positive gravitropism. When exposed to a change in the direction of gravity (e.g. by reorienting seedlings 90°), the direction of new root growth is reoriented toward the new



**Figure 6.** Proposed sequence of gene activity during root hair development in *Arabidopsis*. The top row represents epidermal cells during normal stages of root hair development. Each *RHD* gene is positioned at the point within this pathway where its normal product is initially required. Mutations in *RHD2* result in the production of hairs with the phenotype immediately preceding the gene symbol. Abnormal phenotypes of *rhd1*, *rhd3*, and *rhd4* are drawn below the normal pathway. The relative length of wild-type hairs is roughly twice that indicated.

gravity vector through differential growth of upper and lower portions of the root within the elongation zone. Mutants whose roots are unable to respond normally to gravity have been identified from several species, including tomato (*dgt* mutant; ref 22) and barley (*agr* mutant; ref 23). In later studies, these two mutants were found to be non-responsive to exogenously-applied auxin, in contrast to wild-type roots which display an auxin-induced reduction in root growth.<sup>24</sup> Other workers, specifically interested in the mechanism of auxin action, have recovered several auxin-resistant mutants that were found to display defects in root gravitropic responses. These include the *aux1*,<sup>25</sup> *Dwf*,<sup>25</sup> *axr1*,<sup>26</sup> and *axr2*<sup>27</sup> mutants of *Arabidopsis*. Collectively, these studies strongly implicate auxin as a key hormone in the gravitropic response. Although the precise role of auxin is unclear, the differential root growth following gravistimulation is believed to be the result of an asymmetric distribution of auxin across the root.<sup>28,29</sup> The phenotype of the auxin-resistant mutants is consistent with a defect in an auxin receptor or a component of the auxin signal transduction pathway.

Additional genes involved in the root's gravity sensing and response system have been identified by mutations. Some of these are represented by mutants that do not display an auxin-insensitive phenotype, such as the *Arabidopsis agr1* mutants<sup>30</sup> and the *Zea*

*mays* Ageotropic mutant.<sup>31,32</sup> Starch-deficient mutants have also been useful in exploring the role of amyloplasts in root cap cells during gravity perception. Roots of the *Arabidopsis* TC7 and *Nicotiana sylvestris* NS458 starch-deficient mutants have been shown to respond more slowly to gravity, indicating that starch-containing amyloplasts in the root cap cells are required for full gravitropic sensitivity (refs 33-37; for further discussion, see ref 38).

The genetic basis for the ability of roots to sense and avoid obstacles has also been examined.<sup>10</sup> The approach has been to expose *Arabidopsis* seedlings on Petri plates to a constant obstacle-touching stimulus by tilting at a 45° angle to the vertical. Normal roots exhibit a wavy growth pattern due to periodic, reversible rotation of the root tip as it tries to penetrate the agar surface. Mutants were isolated by their altered growth pattern, which implied that they are unable to sense or respond normally to the obstacle-touching stimulus. Among the six *wav* complementation groups identified, two of the mutants (*wav1* and *wav6*) show no wavy growth and do not display any root tip rotation. Three other mutants (*wav2*, *wav3*, and *wav4*) display abnormal wavy growth patterns with waves of a shorter pitch or irregular angles. One mutant (*wav5*) produces root tip rotation in only one direction, leading to clockwise circles on the agar surface.<sup>10</sup>

Roots from two of the obstacle-avoidance mutants display either no gravitropism (*wav5*) or reduced gravitropism (*wav6*). Complementation tests showed that these mutants are allelic to previously-identified gravitropism mutants *aux1* (*wav5*) and *agr1* (*wav6*). This suggests either that reversible root tip rotation is dependent on some aspect of normal gravity perception and response, or that gravitropism and obstacle avoidance share common components encoded by the *aux1* and *agr1* genes (e.g. elements of a signal transduction pathway). It is currently not known whether root tip rotation is caused by differential growth associated with the asymmetric distribution of auxin.

## Concluding remarks

The genetic analysis of root development has led to the identification of many genes that control root morphogenesis. These include genes affecting several levels of pattern formation: early apical-basal patterning in the embryo, radial patterns of specific cell types in the root, the activity of the root

meristem, and the pattern of cells within specific root cell layers. In addition, genes have been identified that influence root cell differentiation or response to external stimuli. Many of the processes affected by these genes are fundamental to plant development, illustrating again the usefulness of root development as an experimental system for answering basic questions about plant morphogenesis.

Many root development genes remain to be identified and characterized. The recovery of mutant alleles of some of these genes may require different genetic screens to be employed. For example, temperature-sensitive screens may be necessary to identify mutations affecting essential genes whose loss of function would otherwise be lethal. It will obviously be necessary to clone many of the root development genes in order to fully understand the functions of the corresponding products. Work is currently in progress in a number of laboratories aimed at the molecular isolation of these genes through the genetic-based cloning techniques of chromosome walking and T-DNA/transposon tagging. We can therefore expect substantial progress within the next decade toward an understanding of the molecular and genetic basis of root development.

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