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REVIEW ARTICLE

The Development of Plant Roots: New Approaches to Underground Problems

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INTRODUCTION

The roots of terrestrial plants are involved in the acquisition of water and nutrients, anchorage of the plant, synthesis of plant hormones, and storage functions. The development of a root system involves strategies that are common to the development of all plant organs, as well as certain aspects that are unique to roots. Despite the importance of roots and some unusual developmental characteristics, the study of root morphogenesis has not received as much attention as the development of aerial plant organs. This has been particularly true for studies at the molecular and genetic levels. This review is designed to highlight some of the interesting aspects of root development and to describe recent molecular genetic approaches that are likely to advance our understanding of root development.

ROOT MORPHOGENESIS

The Root Apex

Many of the important unanswered questions in root development involve events that occur at the root apex. For example, we know very little about the nature of the stem cells, how cell files are established, how cell numbers and vascular patterning are determined, what controls the organization and size of the meristem, how root hair initials are formed, how cell expansion is regulated, and, perhaps most important, what controls the cell cycle and the planes of cell division. Many of these are general questions that apply equally well to morphogenesis in other parts of the plant. However, several aspects of root morphogenesis serve to simplify the study of these basic questions. First, the root apical meristem is easily accessible (not enclosed the way the shoot apical meristem is), essentially transparent (due to a lack of pigment), and lacks branching primordia. Second, the root as a whole is a simple organ that displays a radial symmetry in the external layers of cells. Third, root morphogenesis normally occurs in a reiterative and uniform fashion, without any major change in the organization of the root apex. Thus, all stages of root development are apparent at all times, and there is nothing analogous to the vegetative to floral conversion that occurs in shoot meristems. Fourth, roots have relatively few differentiated cell types. Finally, the various developmental processes are largely confined to classically defined "zones" along the length of the root, as indicated in Figure 1. These include the meristematic zone (site of cell divisions), elongation zone (cell expansion), and specialization zone (cell differentiation). Although this zonal classification is probably too simplistic (there is overlap in the cellular processes occurring in the various zones), it nonetheless serves to emphasize the spatial separation of these processes in cell files of roots.

To fully understand the morphogenesis of roots, it is necessary to define the organization of the root meristem and determine the fate of cells that emerge from the meristem. One of the most revealing analyses of this type was performed on the root of the water fern Azolla (Gunning, 1982). The precise placement and timing of each cell division were determined and mapped, providing a complete cellular fate map of the root. In higher plants, the characterization of multicellular root meristems led to the discovery of a unique set of cells, the quiescent center, which is located at the center of the root apex but undergoes relatively infrequent cell divisions (Clowes, 1956). The precise function of the cells of the quiescent center and their relationship to the rest of the meristem still need to be defined.

Recent work on Arabidopsis suggests that it represents an excellent system for studying root development in higher plants. Not only is it amenable to molecular genetic analysis (as discussed in later sections), but the basic root morphology is remarkably simple, as shown in Figure 2. Each of the outer layers of cells (epidermal, cortical, endodermal, and pericycle) consists of a single cell layer, and

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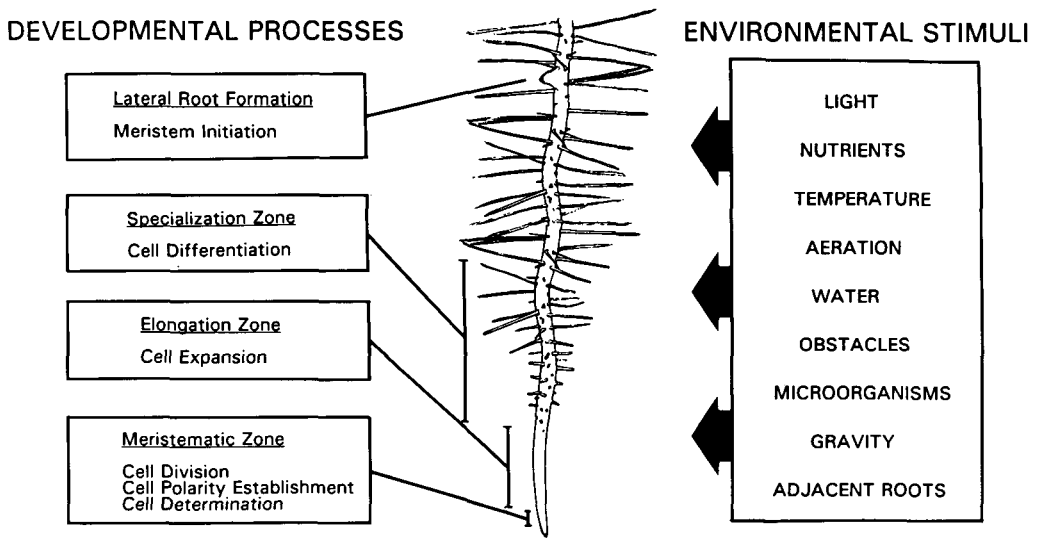

Figure 1. Schematic Outline of Internal Processes and External Factors That Control Root Development.

The classically defined zones of cellular activities are indicated, as are the environmental stimuli that influence root morphogenesis.

there appears to be very little variation in the number of cells (eight) within the cortex and endodermal layers (Figure 2B) (K. Roberts and P. Benfey, unpublished data).

Lateral Roots

Branching in roots differs from branching in stems. Lateral roots do not develop directly from cells in the apical meristem but rather develop from differentiated cells in a special layer (the pericycle) located just below the endodermal layer. Evidence that this process involves rediffer*entiation comes from an analysis of the expression pattern* of the enzyme hyoscyamine 6-β-hydroxylase, which is localized to pericycle cells. Upon induction of lateral roots, expression of the enzyme decreases dramatically (Hashimoto et al., 1991). Not all pericycle cells have an equal probability of giving rise to lateral root primordia; lateral roots are not usually initiated near the root tip (suggesting the presence of an inhibitor diffusing from the tip) and normally are formed from pericycle cells opposite to the xylem elements (Steeves and Sussex, 1989).

The formation of lateral roots is a particularly intriguing aspect of root development because it represents the initiation of a new meristem and may provide clues as to how the primary root meristem arises during embryogenesis. However, important questions regarding lateral root development remain unanswered. The initiation of lateral roots must involve some sort of signal that is perceived by pericycle cells. A single cell may be activated that then recruits others; alternatively, multiple cells may simultaneously perceive the signal. Once the meristem is formed, it must elaborate a root that somehow forces its way through the existing cortex and epidermal tissue and emerges into the external environment. It should be possible to address these issues by identifying genes specifically expressed in these cells and by isolating mutants blocked at different stages of lateral root development.

Plant Hormones

All aspects of root development are profoundly affected by plant hormones, with the strongest effects attributed to auxin, cytokinins, and ethylene (reviewed in Torrey, 1976; Feldman, 1984). Because of the difficulty in interpreting the effect of exogenously applied hormones on internal hormone ratios, there is considerable controversy in the literature as to the relative importance of various growth regulators on root development (Feldman, 1984). Alternative approaches, such as the analysis of transgenic plants in which hormone ratios have been modified in vivo by expression of hormone biosynthetic enzymes (Klee et al., 1987; Medford et al., 1989; Romano et al., 1991) and the characterization of mutants with reduced hormone biosynthesis or altered sensitivity (King, 1988), may help resolve many of the outstanding questions.

Environmental Influences

Although root morphology is guided by a genetic program, the ultimate configuration of a root system under natural conditions is largely determined by environmental factors.

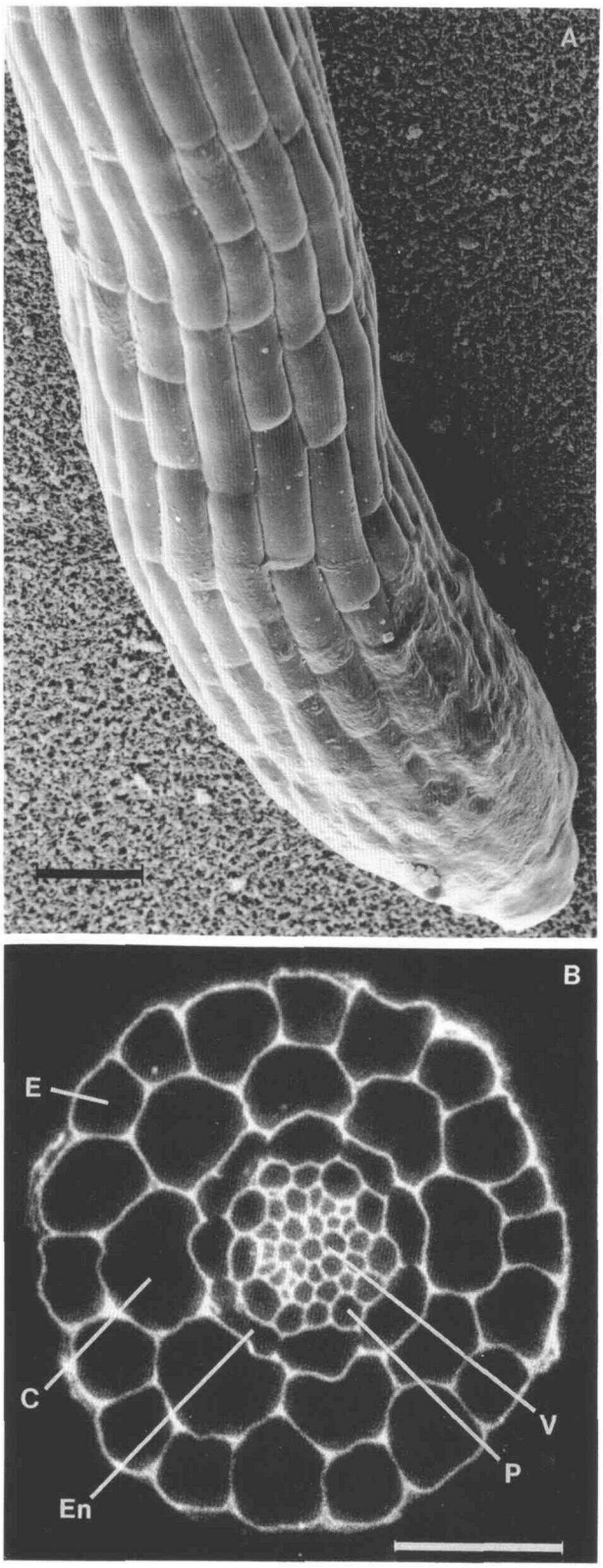

Figure 2. Morphology of Arabidopsis Roots.

(A) Scanning electron micrograph of Arabidopsis root (courtesy of P. Linstead and K. Roberts). Bar = 30 μm.

(B) Cross-section of Arabidopsis root, with cell walls stained by monoclonal antibody against pectin (courtesy of P. Linstead and K. Roberts). The cell layers are epidermal (E), cortex (C), endodermis (En), and pericycle (P) around the vascular cylinder (V). The cell number of the cortex and endodermis (eight cells each) appears to be invariant (K. Roberts and P. Benfey, unpublished data). Bar = 30 μm.

The effects of gravity on root growth have been explored most extensively; roots generally respond in a positive fashion to gravity, with the root cap cells playing a major role in perception (reviewed by Moore and Evans, 1986). Roots also respond to chemical gradients; they proliferate in regions of the soil that contain high concentrations of certain ions, such as nitrate or phosphate (Drew and Saker, 1975, 1978; Fitter et al., 1988). In addition, root growth can be influenced by the soil moisture content, with roots penetrating deeper when soil moisture is low (Coupland and Johnson, 1965) and developing air spaces (aerenchyma) when the soil is waterlogged (Yu et al., 1969). Although roots usually grow in a subterranean environment, light has been shown to affect root extension, gravitropism, and lateral root production in some species (Lake and Slack, 1961; Wilkins and Wain, 1974; Hart and MacDonald, 1980). Finally, the growth of roots can be influenced by temperature gradients (Pahlavanian and Silk, 1988; Fortin and Poff, 1990), mechanical impedence (Barley and Greacen, 1967), aeration (Cannell, 1977), and the roots of adjacent plants (Mahall and Callaway, 1991). The morphological plasticity of roots represents one of the most interesting aspects of root development, and there is a clear need for further exploration of the manner in which external stimuli affect the root's developmental program.

MOLECULAR ANALYSIS OF ROOT DEVELOPMENT

A productive approach to a molecular analysis of root development has been the isolation and characterization of genes that are expressed in the root. Differential screening of cDNA libraries has yielded genes that are specifically expressed in roots (Conkling et al., 1990), preferentially expressed in roots (Evans et al., 1988), or expressed at specific stages of lateral root development (N. Kerk and I. Sussex, unpublished results). In addition, genes expressed during the development of a symbiotic relationship have been identified in legume roots inoculated with Rhizobium (Gloudemans and Bisseling, 1989).

Analysis of gene expression using the β-glucuronidase (GUS) reporter system and RNA blot analyses has revealed root-specific expression of several genes that may play a role in root development. A hydroxyproline-rich glycoprotein gene (HRGPnt3) is specifically expressed in emerging

lateral root cells, leading to the suggestion that its product serves to reinforce the cell wall as the lateral root pushes through the surrounding cells (Keller and Lamb, 1989). The expression of a putative membrane channeling protein gene (TobRB7) in tobacco has been localized to the root meristem and central cylinder region (Yamamoto et al., 1991), and a plasma membrane H^+-ATPase isoform in Arabidopsis has been identified that is preferentially expressed in roots (Harper et al., 1990). Because of the importance of the cytoskeleton in plant morphogenesis (Staiger and Lloyd, 1991), a major goal is the identification and characterization of cytoskeletal protein genes expressed in roots. So far, a β-tubulin isoform has been found to be preferentially expressed in Arabidopsis roots (Oppenheimer et al., 1988), and the distribution of various actin isoforms in soybean root tissues has been characterized (McLean et al., 1990).

The GUS reporter method has also been exploited to identify root-specific expression conferred by certain subdomains of the cauliflower mosaic virus 35S promoter and the petunia EPSP synthase promoter (Benfey et al., 1989, 1990a, 1990b). One of the 35S subdomains binds a transcription factor (ASF1) that is preferentially expressed in root tissue (Katagiri et al., 1989). By working back up the regulatory pathways to the processes that control transcription activation, it should be possible to define the genes regulating early events in root development.

GENETICS OF ROOT DEVELOPMENT

Isolation of Root Mutants

Genotypic variation in root morphology has been described in more than 30 plant species (reviewed by O'Toole and Bland, 1987). Most of these examples, however, involve polygenic variation. The isolation of single-gene mutants traditionally has been hampered by the difficulty in observing large numbers of root systems and by the environmental variation in root morphology. As indicated in Table 1, a relatively small number of single-gene root morphology mutants have been isolated and described. These include a collection of tomato mutants with alterations in root size, lateral root number, and degree of adventitious root growth. These tomato mutants have been useful in differentiating between types of roots (e.g., lateral versus adventitious) and in various physiological studies (Zobel, 1991).

Recently, Arabidopsis has become the organism of choice for studies involving root genetics. The small size of the plant allows large numbers of seedlings to be grown on nutrient agar in vertically oriented Petri plates (>10,000 seedlings/ft^3 of space). Using a dissecting microscope, the roots can be examined as they grow along the agar surface, and variants in root morphology can be easily identified. This method has now been used to isolate a variety of root morphological mutants from chemical, radiation, and T-DNA mutagenesis experiments in Arabidopsis (Okada and Shimura, 1990; Schiefelbein and Somerville, 1990; Feldmann, 1991; R. Williamson, unpublished data; P. Benfey, unpublished data; L. Dolan and S. Poethig, unpublished data; J. Schiefelbein, unpublished data).

Characterization of Root Mutants

Several Arabidopsis root mutants possess gross abnormalities in root length and diameter. Characterization of two mutants with abnormal root diameter revealed that a single cell layer (that differs for each mutant) had undergone aberrant expansion (K. Roberts and P. Benfey, unpublished data). An example of this type of mutant is given in Figure 3. Examination of the abnormally short roots of another mutant revealed an apparent lack of dividing and elongating cells, suggesting that meristematic cells were no longer functioning as stem cells (P. Benfey, unpublished data). Mutants with temperature-sensitive phenotypes have also been isolated, including ones with changes in root cell shape (R. Williamson, unpublished data). Analysis of root morphology mutants should lead to the identification of genes that determine cell size and position within an organ. Because these parameters are a function of the timing of cell division, the orientation of the plane of cell division, and the extent of cell expansion, these genes should be useful in understanding the control of organ morphology.

The genetic control of cell differentiation in roots has been explored through the characterization of root hair mutants in Arabidopsis (Schiefelbein and Somerville, 1990; L. Dolan and S. Poethig, unpublished data). Several mutants possess defects in the initiation of root hairs, implying that the affected genes normally play a role in early stages of cell differentiation and cell polarity. Mutants have also been isolated that affect root hair elongation, a process that normally occurs by expansion at the root hair tip. Because tip growth is a mechanism used by other expanding plant cells, such as pollen tubes, the characterization of this process in root hairs is likely to be of general importance. Root hair mutants should also prove to be useful to address questions concerning the role of root hairs in water and nutrient acquisition (Hochmuth et al., 1985; J. Schiefelbein, unpublished data).

Recently, the genetic basis for the avoidance of obstacles by roots has been explored (Okada and Shimura, 1990). A constant obstacle-touching stimulus was applied to root tips by growing Arabidopsis seedlings on agar plates at a 45° angle. The periodic root-tip rotation patterns that result from this treatment caused wild-type roots to exhibit wavy root growth on the agar surface. Mutants with alterations in the root rotation patterns were isolated and led to the identification of six different loci. Interestingly, two of the six *wav* mutants are allelic with previously

Table 1. Summary of Root Morphology Mutants

Species	Gene	Phenotype	Reference
Arabidopsis	*agr1*	Recessive; altered root gravitropism	Bell and Maher (1990)
Arabidopsis	*aux1*	Recessive; 2,4-D resistant, increased root elongation	Maher and Martindale (1980)
Arabidopsis	*axr1*	Recessive; 2,4-D resistant, reduced lateral roots	Estelle and Somerville (1987)
Arabidopsis	*axr2*	Dominant; IAA[a] resistant, defective gravitropism and root hair growth	Wilson et al. (1990)
Arabidopsis	*Dwf*	Dominant; 2,4-D resistant, lacks lateral roots and root hairs	Maher and Martindale (1980)
Arabidopsis	*rhd1*	Recessive; bulbous root hair initiation	Schiefelbein and Somerville (1990)
Arabidopsis	*rhd2*	Recessive; no root hair elongation	Schiefelbein and Somerville (1990)
Arabidopsis	*rhd3*	Recessive; wavy root hair elongation	Schiefelbein and Somerville (1990)
Arabidopsis	*rhd4*	Recessive; bulging root hair elongation	Schiefelbein and Somerville (1990)
Arabidopsis	*wav1*	Recessive; no root tip rotation	Okada and Shimura (1990)
Arabidopsis	*wav2*	Recessive; altered root tip rotation	Okada and Shimura (1990)
Arabidopsis	*wav3*	Recessive; altered root tip rotation	Okada and Shimura (1990)
Arabidopsis	*wav4*	Recessive; altered root tip rotation	Okada and Shimura (1990)
Antirrhinum[b]		Recessive; reduced root growth rate	Viell-Maek (1978)
Barley	*agr*	Recessive; IAA resistant, increased root elongation rate	Tagliani et al. (1986)
Cotton	*Rc*	Dominant; cracked root	Weaver and Weaver (1979)
Maize	*AGT*	Increased root growth rate	Pilet (1983)
Maize	*rt*	Recessive; few/no secondary roots	Jenkins (1930)
Pea	*agt*	Altered root gravitropism	Olsen and Iversen (1980)
Tobacco	*Rac*	Dominant; NAA[c] resistant, no roots	Muller et al. (1985)
Tomato	*crt*	Recessive; dense root hairs	Hochmuth et al. (1985)
Tomato	*dgt*	Recessive; no lateral roots	Zobel (1973)
Tomato	*ro*	Recessive; no adventitious roots	Butler (1954)
Tomato	*brt1*	Recessive; bushy root with excess lateral roots	Zobel (1975)
Tomato	*brt2*	Recessive; bushy root with excess lateral roots	Zobel (1991)
Tomato	*drt*	Recessive; very short roots	Zobel (1975)

[a] IAA, indoleacetic acid.
[b] No gene designation was assigned to this mutant.
[c] NAA, naphthaleneacetic acid.

characterized root gravitropism genes (*aux1* and *agr1*), implying that proper gravity sensing and response play a role in obstacle avoidance (Okada and Shimura, 1990).

Several auxin-resistant mutants have root growth abnormalities, strengthening the connection between auxin and root development. In Arabidopsis, the *aux1* mutant has roots that display an increased rate of elongation (Maher and Martindale, 1980), the *Dwf* mutant produces more rapidly extending roots that have fewer branches and root hairs (Mirza et al., 1984), the *axr1* mutant has a reduction in lateral root formation (Estelle and Somerville, 1987), and the *axr2* mutant displays a reduction in root elongation and lacks root hairs (Wilson et al., 1990). Interestingly, each of these auxin-resistant mutants also displays defects in root gravitropism. The molecular characterization of the affected genes will be required to understand fully the role of auxin in root development and gravitropism.

FUTURE DIRECTIONS

It is expected that new approaches to the study of root morphogenesis will continue to emerge. One promising system for studying root initiation is the formation of rooty tumors following infection of plant cells by *Agrobacterium rhizogenes*. The *A. rhizogenes rolA*, *B*, and *C* genes that

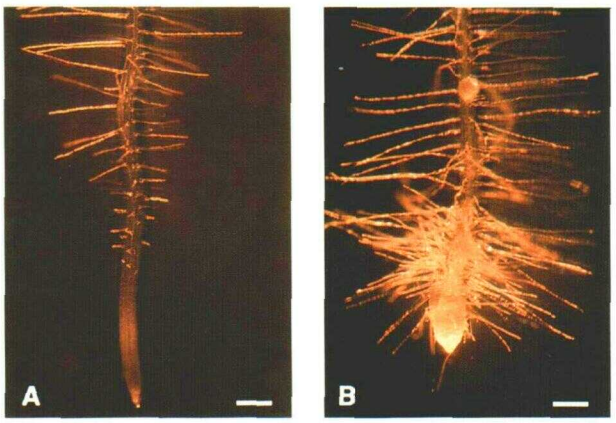

Figure 3. Morphology of Arabidopsis Roots.

(A) Wild type.
(B) Mutant.
The mutant displays aberrant root cell expansion. Bars = 200 μm.

control root proliferation have been characterized and shown to differ in their expression patterns and in their effects on root growth (Schmulling et al., 1988, 1989). *A. rhizogenes* also provides a useful transformation system for studying root-expressed genes because roots containing transferred DNA emerge directly from the site of infection within 2 to 3 weeks (Tepfer and Casse-Delbart, 1987). In addition, the *A. rhizogenes* transformation method or the *A. tumefaciens* root transformation method (Valvekens et al., 1988) could conceivably be used for "shotgun" cloning of DNA fragments (at least from a chromosomal region) to generate wild-type roots from a mutant root population. Another technique that is likely to aid studies in root development is root culture, which enables large amounts of root tissue to be generated in liquid media (White, 1934).

The molecular and biochemical analyses of root-expressed genes should provide useful material for the morphological characterization of root development. For example, promoter-GUS fusion genes with root cell-specific expression patterns are valuable tools for the characterization of root morphological mutants (P. Benfey, unpublished data). Antibodies to cell wall components and membrane proteins are another molecular tool for analyzing root development. For instance, antibodies to arabinogalactan moieties of cell membrane proteins map to a set of cells in Arabidopsis roots that are defined by cell position and appear to provide early markers for developmental events (K. Roberts, unpublished data).

As outlined in this review, roots offer significant advantages in the study of fundamental processes in plant development, and these advantages will allow roots to continue to be used as a model system for molecular genetic studies of post-embryonic development. In the next few years, we are likely to see genes identified and cloned that control root initiation, root morphology, and the differentiation of root cells. Furthermore, the power of genetics to order genes in developmental pathways should greatly enhance our understanding of these processes. In addition, because the root is more than just a model system, the increased understanding of root development should have important applications to agriculture.

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