Primer

Root development

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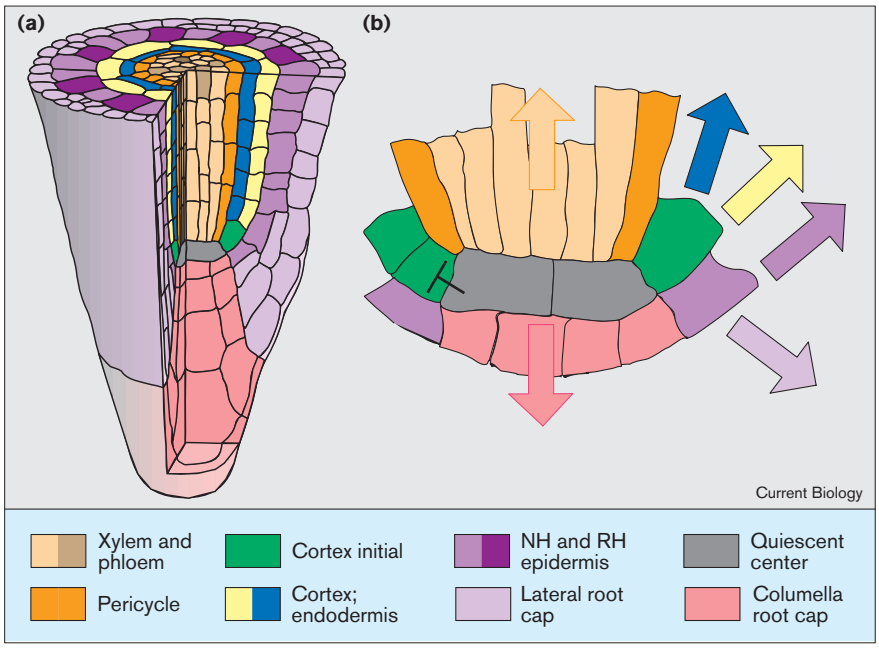
To understand development it is useful to analyze simple systems. Because of the simplicity of its organization, the *Arabidopsis* root is one of the most tractable systems for the study of plant organogenesis. Using primarily molecular genetic approaches, rapid progress has been made in identifying some of the pathways regulating root development. Over the past 10 years, control of radial patterning, specification of epidermal cell types and placement of the root organizing center have begun to be elucidated.

Figure 1



Cell fate in the *Arabidopsis* root meristem. **(a)** Cell types. **(b)** Stem cells (initials) and their direction of cell division.

The simplicity of root development

Arabidopsis roots can be viewed as a set of concentric cylinders. The four outer layers, the epidermis, cortex, endodermis and pericycle surround the vascular tissue in the middle of the root (Figure 1a). The outer epidermis is composed of two cell types, those that form root hairs (RH cells) and those that don't (non-hair or NH cells). Inside the epidermis, the cortex and endodermis layers are each composed of a single cell type. Moreover, there are almost always eight cells in each of these layers.

The pericycle is made up of cells that can initiate the formation of new lateral roots. All cells in the pericycle appear to be capable of lateral root initiation, but under normal circumstances only the pericycle cells nearest the internal xylem poles perform this function. In the center of the root, the vascular tissue has bilateral symmetry with water-conducting xylem on the axis of symmetry and sugar transporting phloem on both sides of it.

Within the growing root, new cells originate at the distal tip in a region known as the meristem. Four sets of initials (the equivalent of animal stem cells) are located around a non-dividing set of cells which form the quiescent center (QC) (Figure 1b). QC cells have a characteristic ultrastructure and express distinct genes. Laser ablation of QC cells leads to increased differentiation of the contacting initial cells, suggesting that the QC acts to maintain the initials in an undifferentiated state. This strategy ensures that after each initial cell division, one daughter cell is disconnected from the QC and allowed to differentiate.

The meristem is protected from the soil by several layers of cells at the root tip which form the root cap. The lateral portion of the root cap is generated from the same set of initials as the epidermis (Figure 1b). The distal part of the root cap, the columella, has its own set of initials as does the vascular tissue. The remaining initials give rise to both the cortex and endodermis (Figure 1b). Each set of initials goes through a stereotyped division pattern to generate its progeny. Because plant cells don't move in relation to each other, the divisions of the root initials give rise to columns or files of cells (Figure 1a). The spatial relationship of cells in a file reflects their age. Younger cells are near the root tip, older cells are higher up in the root. Therefore, all developmental stages are present in every root and anatomy reflects ontogeny.

Radial patterning – signaling inside out

Because of the radial symmetry of the outer root layers, how a three-dimensional structure is formed can be reduced to a one-dimensional problem. The question becomes how cell types are made and specified along a linear axis. To understand how root tissues are formed in the right places with the right identities, mutants were identified that were either missing a tissue or had mis-specified tissues. Among these radial pattern mutants the best characterized, *short-root* and

scarecrow, result in defects in the division and/or specification of the endodermis and cortex. Both the *SHORT-ROOT (SHR)* and *SCARECROW (SCR)* genes encode members of the plant-specific GRAS family of putative transcription factors.

Genetic and molecular data indicate that *SHR* is responsible for specifying endodermis. It is also needed for transcriptional activation of *SCR* which is required for division of the initial daughter cell to form cortex and endodermis. As would be expected, *SCR* is expressed in the initial daughter prior to division. Surprisingly, *SHR* RNA is found not in these cells, but only in the pericycle and vascular tissue internal to these cells (Figure 2a). This argues for a non-cell-autonomous mode of action by *SHR* in order to activate *SCR* transcription. Ectopic expression of *SHR* results in supernumerary layers of cells external to the root vascular tissue. Most of these additional cells exhibit endodermal markers indicating that SHR is sufficient for cell division and cell specification.

This suggests a deceptively simple pathway for radial patterning in which SHR made in the centrally located vascular tissue and pericycle signals the adjacent tissue to divide and specifies the inner layer of the newly divided cells as endodermis (Figure 2a). Shoot phenotypes in *scr* and *shr* mutants as well as expression data suggest that a similar pathway acts in the upper parts of the plant to specify the cell layer surrounding vascular bundles. The nature of the SHR-mediated signaling pathway remains to be identified, as do the upstream regulators and downstream effectors in this pathway.

Circumferential patterning – hairs or no hairs

The epidermis of the *Arabidopsis* root consists of cell files with hair-bearing cells interspersed with files of hairless cells (Figure 1a). The GLABRA2 (GL2) homeobox and WEREWOLF (WER) MYB-type transcription factors are required for the hairless cell fate, whereas the CAPRICE (CPC) protein, with a MYB-like DNA-binding domain but without discernable activator domains, is required for the hair fate. Genetic and molecular data indicate that the WD-40 domain protein, TRANSPARENT-TESTA GLABRA (TTG) acts upstream of GL2 and CPC. Interestingly, the genes for the three transcription factors, GL2, WER and CPC (K. Okada, personal communication) are transcribed predominantly in non-hair cells, suggesting that CPC must promote hair cell formation non-autonomously (Figure 2b). A remarkably similar pathway exists in shoot tissue and is responsible for leaf hair cell (trichome) patterning.

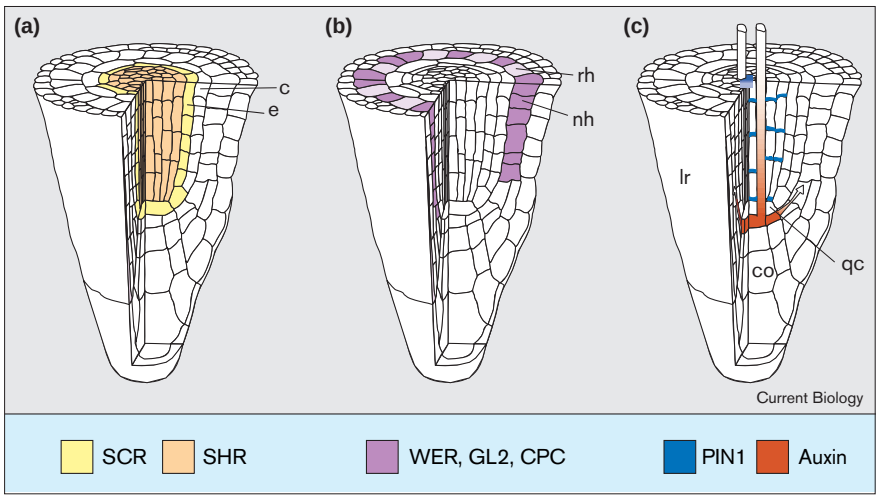
The position of root hair cell files is predictable as hair cells are always found at the junction of two underlying cortical cells (Figure 1a). How this regular spacing of hair cell files is accomplished is still being worked out. Because of the similarity between shoot and root epidermal patterning pathways, it is instructive to make a comparison with trichome spacing in leaves.

Current models suggest that the leaf epidermis starts out as a uniform field of cells within which amplification of small stochastic differences in levels of transcription factors similar to those in the root epidermis results in the observed trichome spacing. Although root hair cell files differ from leaf trichomes in their predictable position above two cortical cells, an initial bias that links the root hair pattern to the underlying cortex could act on a similar stochastic mechanism for root hair patterning. Analysis of the small suite of genes known to play a role in epidermal hair cell patterning will undoubtedly reveal to what extent feed-back loops and amplification steps are instrumental in this simple example of pattern formation.

Proximo-distal patterning: a role for auxin

Laser ablation along with clonal analysis has demonstrated that,

Figure 2



Patterning systems in the *Arabidopsis* root. Distribution of relevant molecules is shown in colour and the specified cell types are named. **(a)** Ground tissue patterning involves the transcription factors SHR and SCR; c, cortex; e, endodermis. **(b)** Epidermal patterning involves the transcription factors WER, GL2, and CPC; rh, root hair bearing cell; nh, hairless cells. **(c)** Distal patterning involves the generation of an auxin maximum and efflux carriers like PIN1. lr, lateral root; co, columella root cap; qc, quiescent center.

despite the regularity of the root cell files, cell lineage is not instrumental in the specification of the distal cell types, QC and root cap. Rather, positional cues appear to be continuously present. What is the distal cue? Recently, the phytohormone auxin has emerged as a likely candidate. A synthetic auxin-response element coupled to the GUS reporter gene displays peak activity just below the QC, and the position of this maximum matches well with direct auxin measurements.

Mutations in genes involved in auxin response and in auxin transport change the magnitude or position of this auxin maximum and display correlated distal pattern defects. Dramatic changes in the maximum, induced by laser ablation and pharmacological inhibition of auxin transport, change proximal into distal cell types and re-direct cell division rates and planes within the root meristem. Thus, in the root tip, the positioning of an auxin maximum seems to be necessary and sufficient to induce the distal pattern.

A large family of proteins implicated in auxin efflux or influx, which show striking polar localization patterns in membranes of specific cell types, is likely to be important for auxin localization. Interestingly, the position of the auxin maximum also influences the polarity of cells, suggesting a feed-back mechanism in which the localization of carrier proteins mediates auxin accumulation and this accumulation in turn influences the localization of the carriers (Figure 2c). The distalizing responses to changes in the auxin maximum are restricted to the root meristem. This may suggest that cell division, itself under auxin control, defines a region of competence for distal patterning.

Establishing the root territory during embryogenesis

Several mutants with embryonic root phenotypes point to an early role for auxin in embryonic root formation, preceding the specification of distal cell types or a meristem. The *auxin-resistant6 (axr6)* and *bodenlos (bdl)* mutants both fail to establish a root pole, and *bdl* as well as *axr6* heterozygotes are auxin-resistant. Furthermore, the *MONOPTEROS (MP)* gene, mutations in which lead to rootless seedlings, encodes a member of the ARF transcription factor family that mediates auxin-induced transcription.

Lastly, mutations in the *GNOM/EMB30* gene eliminate root formation among a variety of other defects. *GNOM* encodes a guanosine nucleotide exchange factor for G-proteins involved in vesicle formation, and *gnom* mutants fail to coordinately localize at least one auxin efflux carrier protein. This may indicate that the inability to form roots observed in *gnom* mutants could at least in part be due to failures in polar auxin transport.

Future prospects

The genetic data suggest an early role for auxins in the establishment of the embryonic root pole. However, the establishment of a root primordium may require a responsive cell population defined in an independent way. Despite elaborate genetic screens, no single candidate gene has emerged with such a role in specifying root identity.

The emerging epidermal patterning and radial patterning gene networks appear to be very similar in roots and shoots. Nevertheless, there are a few root- and shoot-specific genes identified, especially in the epidermal cell specification network. These differences between root and shoot should be caused by differential gene expression. Perhaps factors responsible for these differences have escaped previous genetic screens because of lethal effects or extensive redundancy. More specialized screens and/or genomics approaches may identify the gene(s) critical to root identity.

In addition to providing a tractable model for analyses of plant development, the importance of roots to agriculture is hard to overestimate. Roots are the primary source of water and nutrients, they anchor the plant and frequently serve as storage and/or sites of synthesis of important compounds. Most modern crop breeding programs have been carried out in the presence of externally applied fertilizer. Not surprisingly this has led to many crop plants with sub-optimal root systems. Particularly for farmers in developing countries, attention is beginning to focus on improving roots to better locate and utilize nutrients found in natural soils.

It is not difficult to imagine how changes in radial patterning, root hair location and size, and distal patterning could dramatically modify root function. When coupled with improved knowledge of processes such as lateral root development and cell expansion it may be possible to create plants whose roots are designed for optimum performance in particular soils.

Key references

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