Getting to the root of plant development: the genetics of *Arabidopsis* root formation

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Root development in Arabidopsis thaliana is amenable to molecular genetic analyses because of its simplicity and accessibility. Genetic screens have identified a rich collection of mutants that can be used to address a variety of fundamental questions in plant developmental biology. These mutants have defects in genes that govern organ formation, meristem activity, cell differentiation and response to environmental conditions.

To understand the formation of an organ we need to know the molecular events required to construct a three-dimensional structure with specialized functions. The simpler the structure, the easier the problem, one hopes. Recent progress has focused attention on the *Arabidopsis* root as a model for plant organ development. There are many reasons why this model system is attractive for developmental studies, the primary one being the simple structure of the root, described in detail below. Another important advantage is that roots can be visualized easily by growing *Arabidopsis* seedlings on vertically arrayed petri plates under aseptic conditions, facilitating genetic screens for developmental mutants. Other useful features include the transparency of the root and nature of root growth, which is continuous and rather uniform without any major developmental transitions. Here, we review the structure and development of the *Arabidopsis* root and discuss mutations that should lead to a molecular understanding of the genes that regulate root development.

Embryonic origin of the *Arabidopsis* root

The embryonic phase of plant development lays out the primary body plan and forms two cell populations, known as meristems, at the apical and basal poles of the embryo. It is from these meristems that the shoots and roots that make up the mature plant are formed. The *Arabidopsis* zygote divides asymmetrically to form an apical and a basal cell[1] (Fig. 1a). The basal cell subsequently divides to form the suspensor, whose main function is to provide nutrients to the developing embryo. At the top of the suspensor, a cell known as the hypophysis is also derived from the basal cell (Fig. 1b). The hypophysis goes through a series of stereotyped divisions to form a part of the embryonic root meristem; the rest of the embryonic root is formed from cells that derive from the apical cell (Fig. 1c). Since these two populations of cells of different clonal origin must combine to form the embryonic root, this suggests that cell–cell interactions are required to coordinate the process.

Roots usually grow by a series of transverse cell divisions, producing files of cells. Following these files back to the root tip allows one to make an informed guess as to the progenitors, or initials, of the file. Analysis of mature seeds has shown that there are relatively few cell types in the *Arabidopsis* root[2]. Tracing the cell files has identified the probable initials of each (Fig. 1c). The root meristem is surrounded on all sides by its derivatives. Below the meristem is the root cap, whose cells slough off as the root works its way through the soil so that the meristem remains at a fixed distance from the tip of the root. The root cap cells are derived from two different sets of initials. First, a set of initials gives rise to the central, or columella, root cap cells. A second set of initials develops into the lateral-root cap cells, which surround the columella cells; these initials are also the progenitors of the epidermal cells, which form part of the main body of the root above the meristem. A third set of initials appears to be the progenitor of both the cortex and endodermal

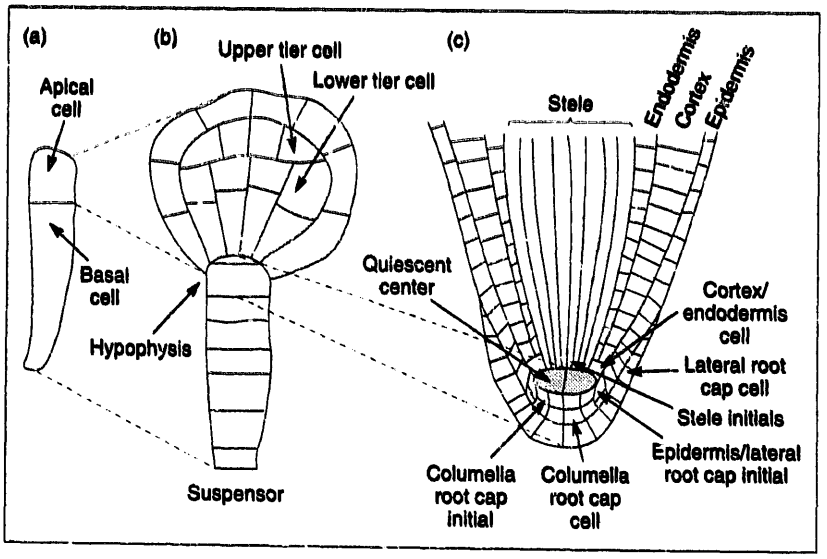

FIGURE 1. Schematic diagram of embryonic root development in *Arabidopsis*. (a) The zygote undergoes an asymmetric division that results in a smaller apical cell and a larger basal cell. The basal cell forms the suspensor and hypophysis, and the apical cell forms most of the embryo proper. (b) The mid-globular stage, in which lower tier cells begin to elongate and no longer resemble upper tier cells. The uppermost derivative of the basal cell, the hypophysis, will divide to form the quiescent center and columella root cap. (c) The embryonic root. To simplify the diagram, individual cells of the stele cell files are not shown. Shading of the quiescent center and the cell walls of the columella root cap indicates that these cells are derived from the hypophysis.

cell layers. Finally, a fourth set of initials gives rise to the stele cells, including the pericycle and vascular tissue.

At the center of the *Arabidopsis* embryonic root lie four cells. From their anatomical position, one might conclude that they are the progenitors of all other cells. However, DNA-labelling studies have shown that the four central cells rarely divide[2]. Similar studies on maize root tips led to the definition of the 'quiescent center' as the population of cells at the center of the meristem that has a very long generation time[3]. It has been proposed that cells in the quiescent center serve as replacements for the more rapidly dividing initials[4]. It appears, therefore, that the four central cells in the *Arabidopsis* embryonic root serve as such a quiescent center (Fig. 1c).

What remains to be demonstrated is that the dynamic relationship of the initials to their progeny is in accordance with their anatomical positions; to this end, clonal analysis studies are underway using a transposable element that interrupts expression of a marker gene (L. Dolan, K. Roberts and B. Scheres, pers. commun.). When the element excises from the marker, the gene is expressed in sectors derived from the cell in which transposition occurred. By analysing these sectors, the clonal origin of the marked cells can be determined.

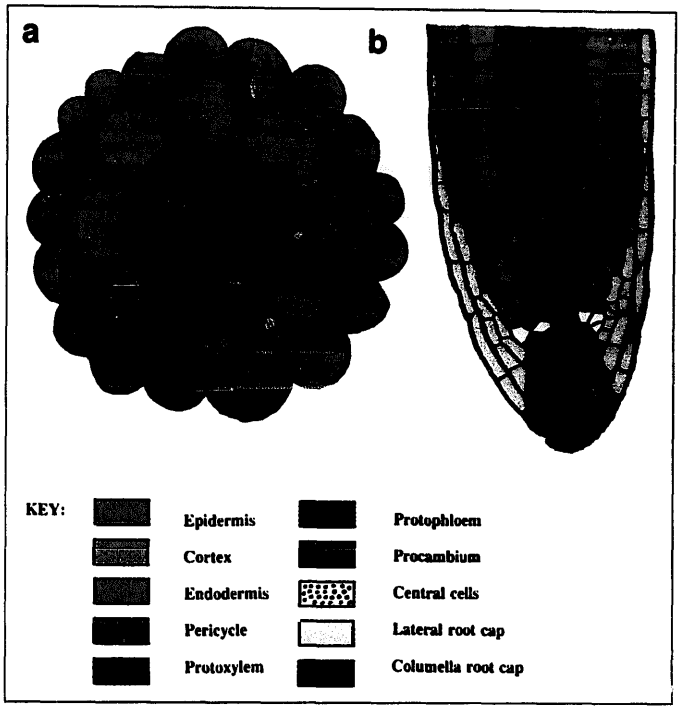

FIGURE 2. Organization of the mature *Arabidopsis* root.
(a) Transverse section through the specialization zone depicting the radial pattern of the different cell types. Scale bar represents 25 μm. (b) Median longitudinal section through the root tip. Sections were stained with antibodies to pectin (a component of the cell wall), reverse-printed and color-coded[2]. (Reproduced, with permission, from Ref. 2.)

Organization of the mature root

The organization of the mature *Arabidopsis* root retains the simplicity of the embryonic root. The radial pattern remains the same, with a single layer of each outer cell type (epidermis, cortex, endodermis and pericycle) (Fig. 2a). The number of cortical and endodermal cells is the same as in the embryonic root, with an invariable number of eight of each cell type in the primary root[2,5]. Within the epidermis there are two types of cell: those with root hairs, and those without. The relative size of the different cell layers is also fairly constant[5]. The organization of the root meristem in the mature root is essentially the same as in the embryo (Fig. 2b).

The apparent lack of cell movement during plant morphogenesis further simplifies the task of dissecting root development. The three-dimensional structure is fashioned by the regulation of three parameters: (i) the timing of cell division; (ii) the orientation of the plane of cell division; and (iii) the orientation and extent of cell expansion. Longitudinal sections of the mature root reveal that regulation of these parameters occurs primarily at the root tip (Fig. 2b). For descriptive purposes, the tip has been divided into three zones: the meristematic zone, which includes the quiescent center and the dividing cells that surround it; the elongation zone, in which cells divide and expand; and the specialization zone, which cells enter as they acquire their differentiated attributes (Fig. 3A). Since root development is continuous, these are, of course, arbitrary distinctions; however, they do serve to highlight the fact that all developmental stages are present at different points on the root axis.

Developmental mutants

Embryonic mutants

Two major processes that affect root development occur in the embryo: first, the establishment of an apical–basal axis; and second, the organization of the root meristem. Mutations that lead to abnormal embryonic root development have been identified. The *gnom* mutation was first identified among seedlings that appeared to lack both shoot and root apical meristems[6]. A detailed study of 24 alleles revealed that they had a range of phenotypes; however, it became apparent that rather than these mutants representing an allelic series, every allele produced the same range of phenotypes. The origin of the defect was traced back to the first zygotic division. In wild-type embryos this division is asymmetric, yielding a smaller apical cell and a larger basal cell (Fig. 1a), but in *gnom* mutant embryos the progeny cells appear to be of similar size[1,6]. Subsequent cell divisions, which are highly stereotyped in the wild type, appear to be variable in the mutant. Variation in the placement of the cell wall during these subsequent divisions is presumed to generate the diversity of phenotype in the mutant. This suggests that correct positioning of the plane of the first zygotic division is a prerequisite for proper formation of the root meristem.

The *monopteros* mutation results in seedlings that have no root, and seedlings carrying strong alleles of the mutation also fail to produce a hypocotyl[6,7]. Shoot

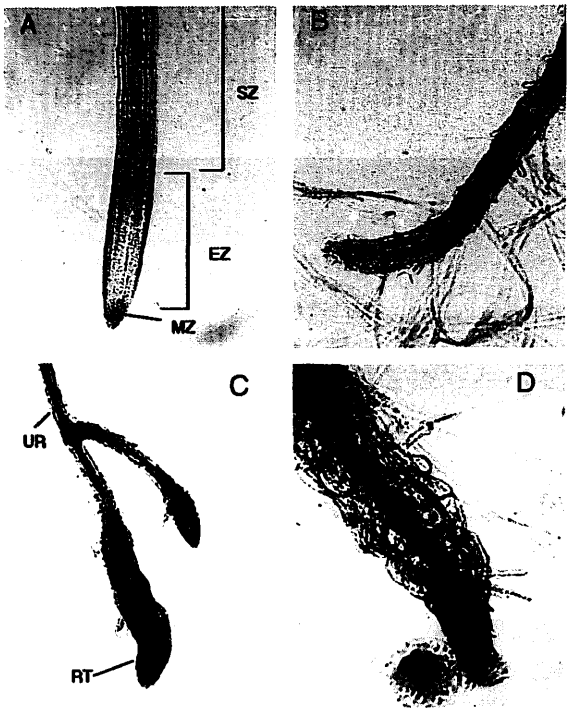

FIGURE 3. Micrographs of whole-mounted wild-type and mutant roots. (A) The wild-type root, showing the meristematic zone (MZ), elongation zone (EZ) and specialization zone (SZ). (B) The mutant *short-root*; note the lack of the elongation zone. (C) The mutant *lion's tail*, which shows abnormal expansion that varies along the length of the root: thus, the upper root (UR) has a relatively normal diameter, while the root tip (RT) is grossly expanded. (D) The mutant *sabre*, which shows relatively uniform abnormal expansion.

apical development appears to be relatively normal. In *monopteros* embryos, abnormal development is first observed at the octant stage, when two processes are under way. The first involves the apical cell, which has divided to give two tiers of cells. Normally, the upper tier will become the cotyledons and the shoot meristem, while the lower forms the hypocotyl and part of the embryonic root and root meristem (Fig. 1b). In *monopteros* mutants, the lower tier of cells fails to undergo the normal pattern of division and elongation, and instead resembles the upper tier. The second process is division of the hypophysis to form the progenitor of the quiescent center and the columella root cap (Fig. 1b). In the *monopteros* mutant, this set of stereotyped divisions does not take place[7]. This suggests that the *MONOPTEROS* gene product might play a role in coordinating interactions between the hypophysis and cells derived from the lower tier to form the embryonic root[7].

The *gnom* and *monopteros* mutations cause gross abnormalities of embryonic root formation. Mutations that cause more subtle changes in the number or pattern of cell layers in the embryonic root might help dissect the molecular events that underlie the simple organization of the root. One such mutation is *bobbit*, which appears to have a specific effect on formation of the root meristem (the quiescent center and cells immediately adjacent) while the surrounding embryonic root appears relatively normal[8]. Another mutation that causes deletion of a cell layer derived from the root meristem is *short-root* (described below).

Morphogenetic mutants

After germination, the three-dimensional structure of the root is realized by division and expansion of cells that emanate from the root meristem. Mutations that disrupt this process would be expected to result in roots of abnormal length or diameter.

The *short-root* mutant was first identified as a plant that has abnormally short roots[5]. The roots initially appear normal, but then completely stop growing. Upon closer examination, there appears to be no elongation and no meristematic zone at the tip of these roots (Fig. 3B). There is evidence of cellular differentiation at the root tip, where there would normally be only undifferentiated cells, indicating that there is a defect in maintaining the self-regenerating capacity of the meristem. This could be considered analogous to defects of animal stem cell populations, such as those seen in aplastic anemia, in which the stem cell population is eliminated and all its derivatives undergo terminal differentiation.

An anatomical analysis of *short-root* mutants revealed that they have no detectable endodermal cell layer. Within the normal endodermis is a suberized region known as the Casparian strip, whose principal function is to force solutes to pass through the plasma membrane (to permit selective uptake), rather than through intercellular spaces. Although the roots of *short-root* mutants appear to lack the Casparian strip, the plants develop quite normally when maintained on nutrient agar. If transferred to soil, their development is stunted, but the plant is fertile[5]. Analysis of mutant embryos indicated that the endodermis is also absent in the embryonic root, suggesting that this is a defect in formation of the radial pattern of the root (B. Scheres, L. Di Laurenzio and P. Benfey, in preparation). The question of whether the defect in radial pattern is causally related to the terminal differentiation of the root meristem should be amenable to molecular analysis.

The degree and direction of cell expansion are critical parameters in determining the final shape of plant organs. It is thought that the external cell wall and the internal cytoskeleton must play important roles in regulating cell expansion[9,10]; however, little is known about the molecular events that control the extent and orientation of expansion in specific tissues. Mutants with abnormal root cell expansion have been identified among plants whose roots have an increased diameter. The degree of root expansion in the mutants *cobra*, *lion's tail* and *pom-pom* varies greatly along the length of the root[5,11] (Fig. 3C). Transverse sections through the expanded regions reveal a different pattern of radial expansion among the root tissues in each of these mutants: in *cobra*, the epidermis is most expanded; in *lion's tail*, the stele shows the greatest expansion; and in *pom-pom*, cell expansion is increased in both epidermis and cortex. These three non-allelic mutants have one feature in common, in that their phenotype is conditional upon the roots growing at a maximum rate. Reducing the rate of root growth by changing the

concentration of sucrose in the medium or growing the plants at lower temperatures results in roots that have relatively normal diameter[5,11]. This suggests that the mutations affect some factor that is limiting for proper cell expansion when roots are growing rapidly, but that functionally redundant processes can substitute for the missing gene products when roots are growing more slowly.

Another type of root expansion mutant isolated had a relatively uniform increase in diameter along the length of the root. Unlike the other expansion mutants, the phenotype of the *sabre* mutant does not depend upon the rate of root growth. Expansion is again greatest in one cell layer, the cortex[5] (Fig. 3D). The *SABRE* gene has been isolated and work on its molecular function is in progress (R. Aeschbacher and P. Benfey, in preparation).

Root expansion mutants have also been isolated in a screen for temperature-sensitive phenotypes. Three non-allelic mutants, *rsw1*, *rsw2* and *rsw3*, have a wild-type phenotype at 18°C, but have grossly abnormal radial swelling when transferred to 31°C (Ref. 12). With the exception of abnormal epidermal cell swelling seen in *rsw3*, it is not yet known whether the swelling in these mutants affects specific tissues. Tests are under way to determine whether the *rsw* mutants are allelic with the expansion mutants described above.

Differentiation mutants

All newly generated plant cells undergo a series of specific steps that lead to formation of fully differentiated cells. The initial step is believed to involve the specification of cell fate, whereby a cell is directed to follow a particular pathway of differentiation. In later steps, the shape and biochemical characteristics of that cell type become established. In the *Arabidopsis* root, the differentiation of epidermal cells has been studied in the greatest detail, and many mutants that affect steps in this process have been identified.

Newly formed root epidermal cells are faced with an initial 'either/or' choice. They can differentiate into root-hair cells (cells that produce a long tubular extension), or into epidermal cells that lack root hairs. The mechanism that controls the fate of root epidermal cells in the family Brassicaceae (of which *Arabidopsis* is a member) appears to be influenced by cell position. Root-hair cells are always located at the junction between adjacent cortical cells[2,13]. Recently, mutants that have abnormalities in the specification of epidermal cell fate, producing roots that contain too many or too few root-hair cells, have been identified (Fig. 4c) (M. Galway and J. Schiefelbein, unpublished).

The first outward sign of root-hair formation is the generation of a swelling, or bulge, at the lower end of the elongated epidermal cell. Interestingly, mutations that alter the position where the root hair emerges from the epidermal cell have yet to be identified. Mutants that have alterations in the size of the initial bulge have been isolated: both the *rhd1* mutant[14] and the *reb1* mutant[12] produce an excessively swollen bulge. This phenotype may reflect a defect in regulated degradation of the cell wall, or its localized expansion.

The root hair itself grows by addition of cell wall material at its tip, a special sort of polarized cell expansion known as tip growth. Several mutations, including *rhd2*, *rhd3*, *rhd4* and *tip1*, affect this process in *Arabidopsis*. The *rhd2* mutants produce root hairs that fail to elongate, indicating a lack of tip growth (Fig. 4b), whereas *rhd3*, *rhd4* and *tip1* mutants produce abnormally shaped root hairs (Fig. 4d), reflecting aberrant control of the orientation or the extent of tip growth[14,15]. The *tip1* mutation is unusual in that it also disrupts the growth of pollen tubes, another type of plant cell that shows tip growth[16].

Stimulus-response mutants

Roots, like other plant organs, have a growth pattern that is remarkably plastic. The ultimate size and configuration of a root system is greatly influenced by environmental conditions: roots are known to respond to gravity (gravitropism), light (phototropism), physical obstacles (thigmotropism), water (hydrotropism), temperature (thermotropism) and certain ions (chemotropism). *Arabidopsis* mutants have been isolated whose roots have an altered

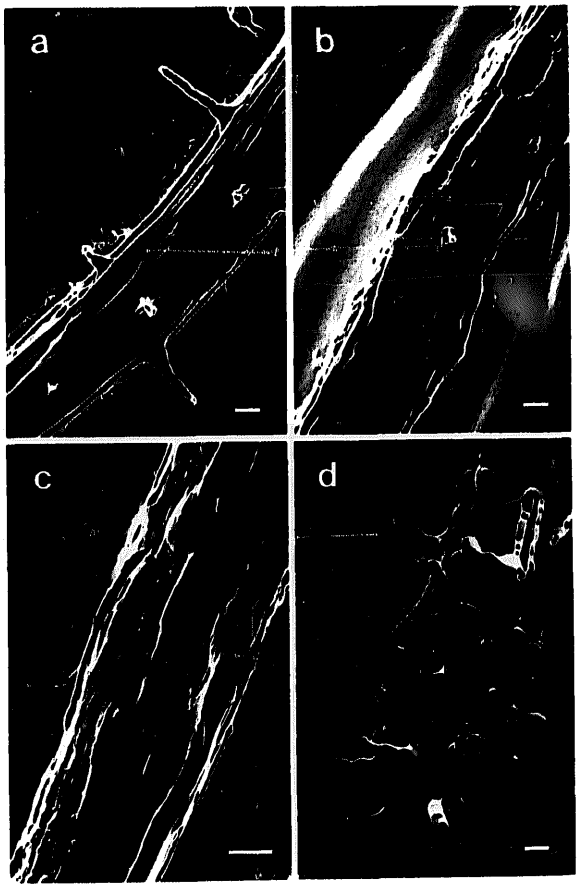

FIGURE 4. Development of root hairs in wild-type and mutant *Arabidopsis* plants. Each scanning electron micrograph shows a segment of a root in the region where root hairs normally develop. (a) A wild-type root (Columbia ecotype). (b) *rhd2*, a 'stubby' root-hair mutant. (c) A root-hair initiation mutant, with no sign of hair formation. (d) *rhd4*, a root-hair elongation mutant, whose hairs have an abnormal morphology. Scale bars represent 20 μm.

capacity to perceive or respond to gravity, light and obstacles.

Many stimulus-response mutants with altered root gravitropism have been identified. While wild-type *Arabidopsis* roots respond positively by growing toward the gravity vector, gravitropism mutants do not respond, or respond abnormally. At least five loci are involved in the gravitropic response: *aux1* (Ref. 17), *dwf* (Ref. 17), *agr1* (Ref. 18), *axr1* (Ref. 19) and *axr2* (Ref. 20). Four of these mutants, *aux1*, *dwf*, *axr1* and *axr2*, are also resistant to auxin, lending support to the hypothesis that auxin is involved in transport or transduction of the gravitropic 'signal' from the root cap to the elongation zone[21].

Root phototropism mutants have also been isolated. Wild-type roots typically grow away from the light source. This response is altered in two *Arabidopsis* mutants, *rpt1* and *rpt2* (Refs 21, 22), whose roots continue to grow vertically when illuminated from one side. These loci appear to be specific for root phototropism, since gravitropism and shoot phototropism are unaffected in these mutants.

An elegant genetic screen was used to identify *Arabidopsis* mutants whose roots respond abnormally to obstacles. To mimic the effect of a physical obstacle, Okada and Shimura[23] tilted a petri plate at a 45° angle to the vertical. Wild-type roots on the surface of the hard agar medium in the plate grow toward the gravity vector, but are unable to penetrate the medium. The roots rotate, trying to avoid the 'obstacle'. After they have rotated in one direction, the gravitropic response causes the roots to rotate back the opposite way. This leads again to the obstacle-avoidance response (root rotation), and this periodic rotation results in a 'wavy' root phenotype. Six mutants were isolated that display an altered root growth pattern, and these were designated *wav1–wav6* (Ref. 23). Not surprisingly, some of these mutants showed alterations in gravitropism. The *wav5* and *wav6* mutations are allelic with the gravity-response loci *aux1* and *agr1*, respectively.

Prospects

The work on *Arabidopsis* root development is all quite recent. There is a growing realization that the combination of the structural simplicity and ease of genetic analysis in this system has enormous potential in understanding plant organogenesis. Already, genes have been identified that influence embryonic root formation, root cell division and expansion, cell differentiation and response to environmental stimuli. Once clonal analysis of the root is completed, it may not be far-fetched to compare the *Arabidopsis* root to another favored genetic system, the nematode *Caenorhabditis elegans*.

Many aspects of root development remain to be explored. Among these are developmental changes in response to environmental stimuli, such as gradients of inorganic compounds, drought and water stress, and bacterial and other pathogens. Another challenge is the identification of genes that play important roles in root development, but would be missed in a genetic screen because they are functionally redundant or because their mutation is lethal in early embryos; one means of identifying such genes is through enhancer- or promoter-trapping strategies.

The fruit of these analyses should be a better understanding of such fundamental processes as the regulation of cell division, cell expansion and radial pattern organization. The genes identified might be analogous to those that regulate formation of other plant organs, and may well reveal insights into the processes that regulate organogenesis in other organisms.

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References

1 Mayer, U., Buttner, G. and Jurgens, G. (1993) *Development* 117, 149–162
2 Dolan, L. *et al.* (1993) *Development* 119, 71–84
3 Feldman, L.J. (1984) *Am. J. Bot.* 71, 1308–1314
4 Barlow, P.W. (1976) *J. Theor. Biol.* 57, 433–451
5 Benfey, P.N. *et al.* (1993) *Development* 119, 57–70
6 Mayer, U. *et al.* (1991) *Nature* 353, 402–407
7 Berleth, T. and Jurgens, G. (1993) *Development* 118, 575–587
8 Scheres, B. *et al.* in *Molecular-Genetic Analysis of Plant Development and Metabolism* (Puigdomenech, P. and Coruzzi, G., eds), Springer-Verlag (in press)
9 Roberts, K. (1989) *Curr. Opin. Cell Biol.* 1, 1020–1027
10 Carpita, N.C. and Gibeaut, D.M. (1993) *Plant J.* 3, 1–30
11 Hauser, M-T. and Benfey, P.N. in *Molecular-Genetic Analysis of Plant Development and Metabolism* (Puigdomenech, P. and Coruzzi, G., eds), Springer-Verlag (in press)
12 Baskin, T.I. *et al.* (1992) *Aust. J. Plant Physiol.* 19, 427–438
13 Bunning, E. (1951) *Planta* 39, 126–153
14 Schiefelbein, J.W. and Somerville, C. (1990) *Plant Cell* 2, 235–243
15 Schiefelbein, J.W., Shipley, A. and Rowse, P. (1992) *Planta* 187, 455–459
16 Schiefelbein, J.W., Galway, M., Masucci, J. and Ford, S. (1993) *Plant Physiol.* 103, 703–711
17 Mirza, J.I., Olsen, G.M., Iversen, T-H. and Maher, E.P. (1984) *Physiol. Plant.* 60, 516–522
18 Bell, J.C. and Maher, E.P. (1990) *Mol. Gen. Genet.* 220, 289–293
19 Estelle, M.A. and Somerville, C. (1987) *Mol. Gen. Genet.* 206, 200–206
20 Wilson, A.K., Pickett, F.B., Turner, J.C. and Estelle, M.A. (1990) *Mol. Gen. Genet.* 222, 377–383
21 Okada, K. and Shimura, Y. (1992) *Cell* 70, 369–372
22 Okada, K. and Shimura, Y. (1992) *Aust. J. Plant Physiol.* 19, 439–448
23 Okada, K. and Shimura, Y. (1990) *Science* 250, 274–276

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