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The *Arabidopsis* root as a model to study plant development

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Abstract With its straightforward amenability to genetic approaches, *Arabidopsis thaliana* proves to be a powerful system to study biological processes in plants. The root of *Arabidopsis* has several additional features which make it a good model system to study developmental processes. It has a largely invariant ontogeny and fixed cellular organization. Continuous cellular differentiation in the root meristematic region allows monitoring of developmental processes after embryogenesis. The *Arabidopsis* root is also amenable for experimental manipulation. The simplicity of its organization allows large-scale mutant screens covering several aspects of root formation and growth. Experimental manipulations have uncovered the existence of positional cues important for pattern formation. Together with the several classes of mutants and the ongoing identification of genes these findings contribute to a better understanding of developmental processes at the cellular level. © Elsevier, Paris.

Key words Root, pattern formation, laser ablation, cell division, cell expansion, environment, *Arabidopsis*.

Abbreviations CLSM, confocal laser scanning microscope; GFP, green fluorescent protein; GUS, ß-glucuronidase.

Introduction

It is beyond doubt that development is complex. Therefore, the use of model organisms that allow optimal experimental strategies is common practice among developmental biologists. A major criterion for selecting animal model systems like the fruit fly, the worm *Caenorhabditis elegans*, the mouse and the zebrafish has been the straightforward applicability of genetic analysis. Genetics (both "forward", starting from a mutant phenotype, and "reverse", starting from a known gene sequence) allows one to study development by interfering "from within" by mutation. This approach has proven to be powerful for the identification of genes involved in development. Once gene products are identified, biochemical and physiological studies can yield knowledge of the relevant molecular mechanisms. The relatively recent successes in the identification of gene products involved in development of *Arabidopsis thaliana, Antirrhinum majus* and *Zea mays* demonstrate that the study of plant development is heading in the same direction. A major role is played by genetic approaches at present, but these do not operate in isolation as they either prelude or go hand in hand with detailed physiological and biochemical studies.

It is, however, not only the applicability of genetics that determines the experimental ease with which processes can be studied in detail. The nematode *C. elegans* serves to illustrate that two other features of organisms are extremely useful for developmental analysis. Firstly, a constant cellular organization and cell lineage (relation between mother and daughter cells) enables one to identify cells by position and to accurately predict the destiny of any cell in a developing organism (Sulston and Horvitz, 1977). Secondly, transparency of developing structures allows physical manipulation of cells. Such an ability to interfere with development is of extreme importance in formulating or testing of hypotheses on cellular interactions. In *C. elegans*, the knowledge of cell lineage has facilitated the identification and analysis of mutant phenotypes (*e.g.* Horvitz and Sternberg, 1991). The transparency has been utilized to manipulate cells by reshuffling (*e.g.* Wood, 1991; Goldstein, 1992), by destruction of cells with a laser beam (*e.g.* Sulston and

Horvitz, 1977), and by microinjection (*e.g.* Evans *et al.*, 1994; Hunter and Kenyon, 1996).

Studies of the past five years have revealed that the *Arabidopsis* root has a for plants uncommonly constant cell lineage. Moreover, this organ is amenable to experimental analysis by virtue of its small size and transparency. Here, we review recent studies on *Arabidopsis* roots that exploit these features, often in combination with genetic screens for root mutants. This work has increased our understanding of pattern formation and control of cell proliferation in roots, but it also begins to address complex aspects of development which are specific to plants, such as cell expansion and response to the environment.

Cells of the *Arabidopsis* root meristem have predictable fates

The mature part of the *Arabidopsis* root has an uncomplicated radial organization in which each of the cell layers can be readily recognized by their morphological characteristics. From outside to inside, concentric layers of epidermis, cortical parenchyma ("cortex" hereafter) and endodermis with almost constant cell numbers encircle the stele tissue (*fig.* 1 A). Within the stele, the pericycle encloses a simple diarch vascular system consisting of two phloem and two xylem poles (Dolan *et al.*, 1993).

The meristem adds new cells to the mature part of the root, and the resulting regular cell files match and extend the earlier formed pattern of mature cells (*fig.* 1 B). The basal-most cells of these cell files, termed initials, act as stem cells. They produce basal daughter cells that will remain initial-like and proximal daughters, which generate cells that differentiate upon displacement from the meristem. The sequence of cell division of the initials and their daughters is essentially invariant, which leads to a fixed contribution of the meristem cells to the different tissues (*fig.* 1 C).

The distal-most columella initial cells and the epidermal initials give rise to the root cap, of which the outer cells detach in time from the main root body. Due to the disappearance of the outer cap layers the initials remain at the same distance from the root apex. All initials surround a group of four non-dividing central cells termed the "quiescent centre" (Dolan *et al.*, 1993). Under certain growth conditions these cells can be mitotically active to contribute to the cortical cell layer (Rost *et al.*, 1996). The extent of violation of the lineage relationships depicted in figure 1 C is investigated by genetically marking the progeny of single cells, and analysis of their contribution to tissues. Such a "clonal analysis" corroborates the notion of strict lineages in the *Arabidopsis* root (Dolan *et al.*, 1994; Scheres *et al.*, 1994). The predictability of the fate of individual cells in the *Arabidopsis* root greatly facilitates the interpretation of genetic and experimental analyses.

The embryonic ontogeny of the *Arabidopsis* root is known

The developing *Arabidopsis* embryo undergoes stereotyped cell divisions, which enables one to assess the contribution of embryonic cells to the different organ primordia (Mansfield and Briarty, 1991; Jürgens and Mayer, 1994). At the octant stage of embryogenesis in *Arabidopsis*, three domains can be defined (*fig.* 2). The apical domain forms the shoot apical meristem and the majority of the cotyledons, the central domain gives the hypocotyl and the majority of the root and the basal domain forms two cell types within

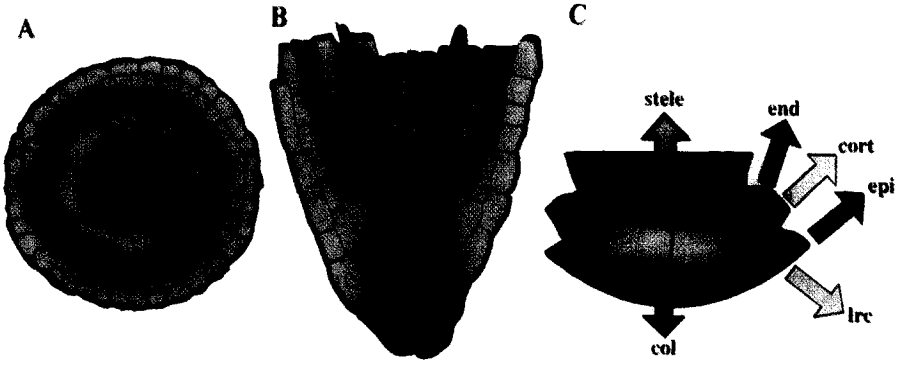

Figure 1. *Schematic representation of tissues and cell types in the* Arabidopsis *root.* **A**, transverse section. **B**, longitudinal section. **C**, blow-up of initials surrounding quiescent centre (grey), arrows indicating direction in which initial cells add new cells to files. Note that cortical initials (green) and epidermal initials each give rise to two different cell types.

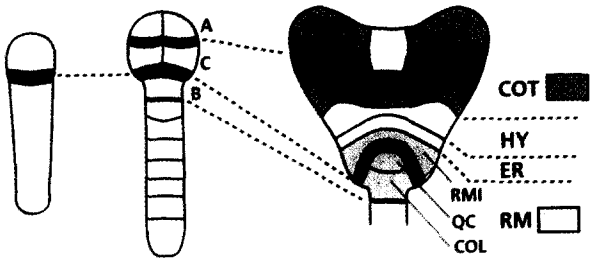

Figure 2. *Fate map of the* Arabidopsis *embryo.* Left to right: 1-cell, 8-cell and heart stage embryos. Bold lines: divisions separating apical (**A**), central (**C**) and basal (**B**) embryo regions (Jürgens, 1995). The basal embryo region is the hypophysis. Cell groups which give rise to seedling structures are indicated in the heart stage embryo. SAM, shoot apical meristem; COT, cotyledons; HY, hypocotyl; ER, embryonic root; RM, root meristem; RMI, root meristem initials; QC, quiescent centre; COL, columella root cap.

the distal region of the root the columella root cap and the quiescent center.

Analysis of the contribution of genetically marked embryonic cells to the root and hypocotyl has revealed that three lineage relationships are essentially invariant (Scheres *et al.*, 1994). Firstly, the hypophysis, constituting the basal domain of the octant embryo, will give rise to the quiescent centre and the columella root cap only (*fig.* 2). Secondly, the separation of the protoderm, ground tissue and procambium is completed at the globular stage of embryogenesis, and these concentrically arranged layers invariantly give rise to epidermal- cortical- and stele tissues, respectively. A third constant relationship is that the cells which flank the hypophysis-derived cells at the late heart stage of embryogenesis become the root meristem initials (*fig.* 2).

The analysis of sectors has, however, also revealed variability in the contribution of embryonic regions to seedling structures. The hypocotyl/root boundary within the central domain, for example, only roughly coincides with a subdivision of the lower tier occurring at the early heart stage of embryogenesis. This subdivision is therefore not sufficient to separate cell fates. Border cells from either the upper or lower region are able to adopt the fate of the neighboring region, which indicates that the sharp boundary between root and hypocotyl is fixed at later stages of development. The root boundary is not an exceptional case of non-identity of embryo domains and organ primordia, as many cells derived from the central region contribute to the cotyledons (*fig.* 2). Such variable contributions reveal that the early subdivisions of the embryo do not segregate cell fates associated with the organ primordia.

In conclusion, root development during normal embryogenesis involves a rigid sequence of cell divisions and, with the exception of the root/hypocotyl boundary region, the origin of cells at a given developmental stage is known.

Experimental manipulation: control of cell fate and cell differentiation

In the nematode *C. elegans*, selected cells can be destroyed with a laser beam ("laser ablation"). Experiments relying on this technique have led to the discovery that, despite the rigid cell lineage, cell position was instrumental in determining the fate of many cells (*e.g.* Sulston and White, 1980). Living *Arabidopsis* roots, with their regular cell lineage relationships, can be imaged using confocal laser scanning microscopy (CLSM) (*fig.* 3). This has allowed the application of

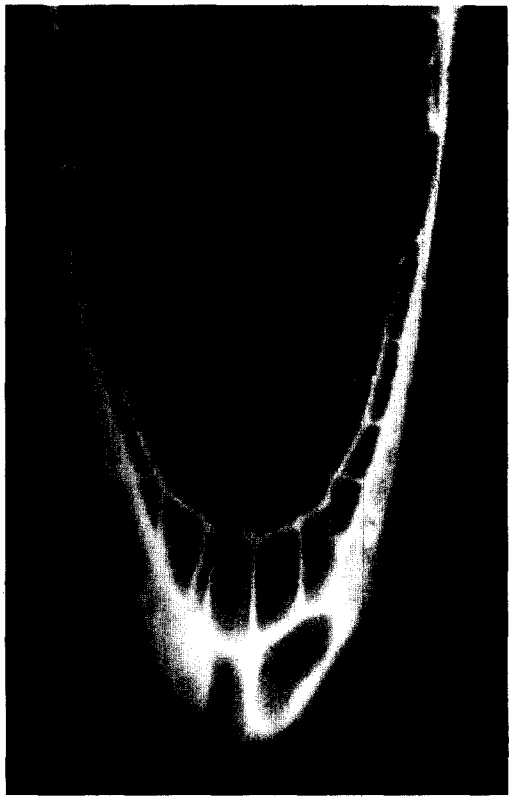

Figure 3. *Confocal laser scan depicting the propidium iodine stained root tip of a living* Arabidopsis *seedling.* The dye accumulates between cells and visualizes the cellular organization. Laser ablation is performed by parking the laser beam on a defined cell. Entry of the dye inside cells marks succesful ablation experiments.

laser ablation to the study of root development. One major conclusion was that, like in *C. elegans*, position is of major importance in determining cell fate (van den Berg *et al.*, 1995).

In plants, cell lineage is generally variable. For example, plasticity of shoot meristem development is evident from sector analysis and from surgical experiments (Poethig, 1987; Steeves and Sussex, 1989). The predictable cell fates in the *Arabidopsis* root therefore do not appear to arise from an exceptional mechanism of development, but rather they reflect precise cell division sequences that conserve the spatial organization of cells. Taken together, the root meristem provides us with a unique combination of a nearly constant cell lineage, facilitating the analysis of experiments, and the flexible position-dependent mode of development that is considered the hallmark of plant development.

Laser ablation experiments have also demonstrated that cortex initial cells perform the appropriate asymmetric divisions giving rise to cortex and endodermis only when in contact with more mature cells within the cortical cell layer. This has led to the suggestion that positional information in the root meristem is provided by "top-down signaling": more mature cells act as a template to guide cell fate of the root meristem initials (van den Berg *et al.*, 1995). The molecular nature of the signals involved is so far unknown. Elegant experiments on embryos of the brown alga *Fucus spiralis* have created some precedence for a role of the cell wall in signaling cell fate (Berger *et al.*, 1994). On the other hand, studies on the distribution and conductance of plasmodesmata in the *Arabidopsis* root tip reveal predominant plasmodesmatal connections within cells of the same tissue layers, which would be in line with cytoplasmic transmission of positional information (Zhu, T., Lucas, W. J. and Rost, T., personal communication). *Arabidopsis* roots can be micro-injected, and such experiments have demonstrated cytoplasmic isolation of fully differentiated cells (Duckett *et al.*, 1994). This observation suggests that cytoplasmic connections may transmit positional information only in the meristematic region where cells are not yet completely differentiated.

The mechanism by which the meristem retains undifferentiated cells has also been studied in the *Arabidopsis* root. Ablation of quiescent centre cells has revealed that they inhibit differentiation of the columella and most likely all initial cells. They do so through signals which act at the single-cell range and are possibly contact-dependent (our unpublished data).

In conclusion, the ability to destroy single cells in the *Arabidopsis* root meristem has provided us with evidence for positional information guiding cell fate, with a model on how this information is transmitted, and with evidence for signals, emanating from the quiescent centre, which control cell differentiation.

Landmarks allow identification of developmental mutants

The developmental systems in which genetic analysis has been successfully applied all possess easily distinguishable markers ("landmarks") for development. The larval denticle belts, which are characteristic for each segment in fruit flies, form a classical example of conspicuous traits aiding in the identification of developmentally relevant genes (Nüsslein-Volhard and Wieschaus, 1980). The dependence on landmarks is also evident when one considers the area of major progress in plant development: the easily distinguished floral organs with their many distinct features have enabled the identification of many relevant floral mutants (Weigel and Meyerowitz, 1994).

Despite its structural simplicity, the *Arabidopsis* root contains many recognizable markers which can be utilized for genetic screens (Benfey and Schiefelbein, 1994). However, these landmarks are not always easily scored under the dissecting microscope which is the method of choice in most large-scale genetic screens for developmental mutants. Root hair morphology, root cell elongation, and root meristem activity have been useful traits to isolate specific mutants (see below), but hitherto the identification of pattern mutants has been somewhat indirect. Mutants with radial as well as apical-basal pattern defects have been mainly pre-selected by a secondary criterion: root growth (Benfey *et al.*, 1993; Scheres *et al.*, 1995; our unpublished data). This is a biased way of pre-screening, which presupposes that a growth defect accompanies the primary defect. Consequently, patterning mutants with normal growth and embryonic lethal pattern mutants will be missed. Indirect pre-screening can be avoided in future by two different approaches. Firstly, once a small set of genes is identified which is specifically involved in a particular developmental pathway, enhancer or suppressor screens may allow further analysis of this pathway. Secondly, transgenic lines with marker genes that highlight a particular cell type or region in the developing organism, facilitates

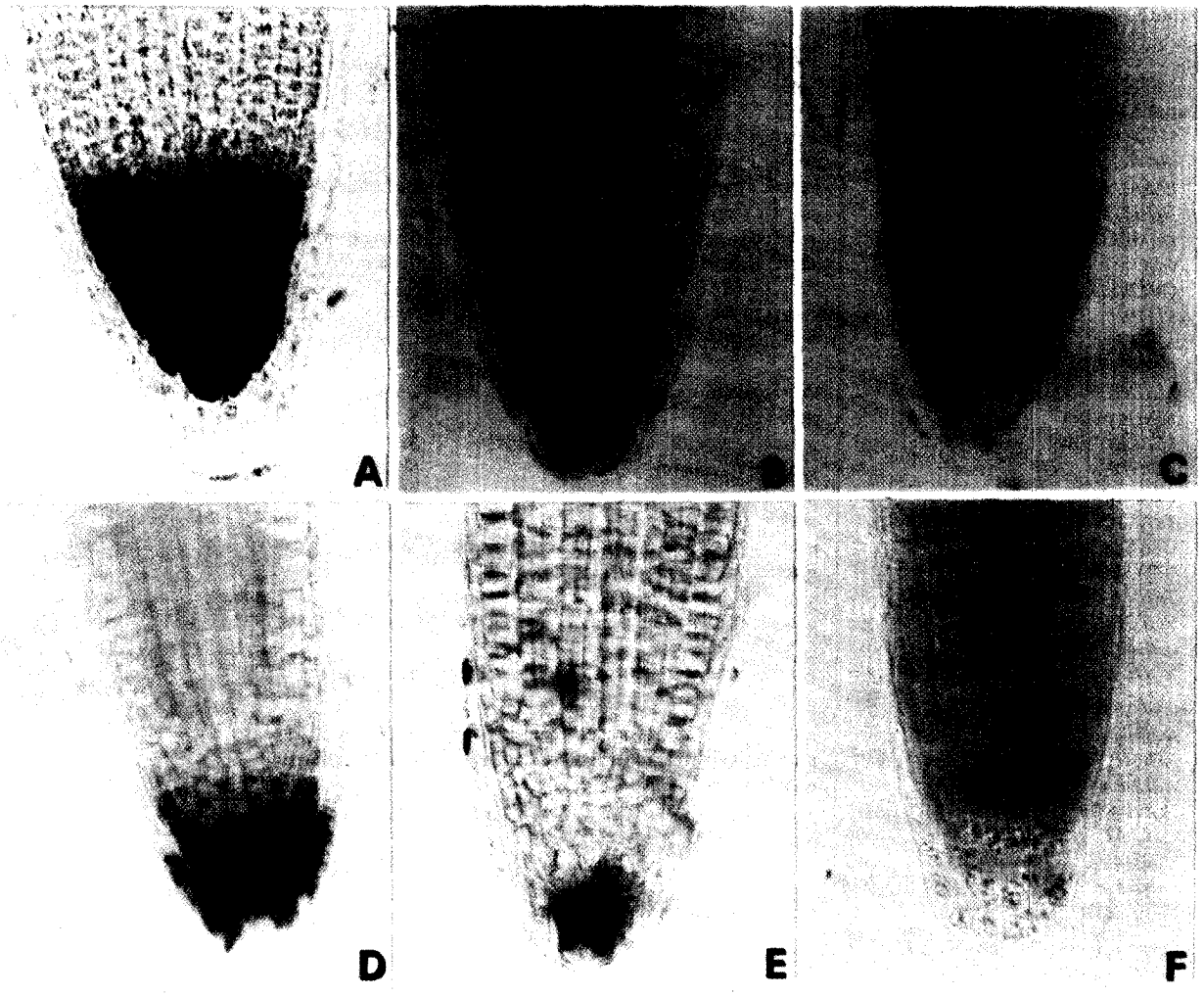

Figure 4. *X-Gluc stained roots from transgenic* Arabidopsis *containing β-glucuronidase (GUS) fusions which mark specific regions in the root tip.* **A**, complete root cap marked by 35S-B2::GUS fusion (Benfey *et al.*, 1990). **B**, columella root cap marked by promoter trap pMOG533-1027 (Goddijn *et al.*, 1993). **C**, lateral root cap marked by enhancer trap LRC244 (Malamy and Benfey, 1997). **D**, outer layers of complete root cap marked by pMOG533-649. **E**, outer layer of columella root cap marked by pMOG533-174. **F**, quiescent centre and surrounding intials marked by pMOG533-124.

the identification of less obvious phenotypes. Such transgenic lines are now available (*fig.* 4; Malamy and Benfey, 1997; Topping and Lindsey, 1997; our unpublished data). The combination of enhancer trap constructs with selectable transposition allows large-scale isolation of lines with relevant expression patterns (Sundaresan *et al.*, 1995). Currently available promoter and enhancer trap lines can be used for mutagenesis experiments to identify genes involved in setting up specific expression patterns. Especially promising for this purpose are recently described enhancer traps based on Green Fluorescent Protein (GFP) fusions (Haseloff *et al.*, 1997). The GFP protein can be visualized directly in living roots, without adding toxic chemicals.

Genetic analysis

Over the past years, the *Arabidopsis* root has been the subject of a number of genetic studies aimed at defining genes involved in a large variety of processes (*tab.* 1). Below we will briefly highlight a selection of the identified mutants/genes, grouped into four broad functional categories: pattern formation, cell division, cell expansion and environmental responses. Our

Table 1. Arabidopsis *genes and the phenotype they display in roots.*

Gene	Phenotype	References
Pattern formation		
MONOPTEROS	aberrant axialization of provascular tissue	Berleth and Jürgens, 1993
MÖWE	reduced hypocotyl, missing root	Berleth *et al.*, 1996
BASAL DELETION	reduced hypocotyl, missing root	Berleth *et al.*, 1996
HOBBIT	no root meristem	Scheres *et al.*, 1996
BOMBADIL	reduced hypocotyl, missing root	Scheres *et al.*, 1996
ORC	defective root meristem	Scheres *et al.*, 1996
GREMLIN	defective root meristem	Scheres *et al.*, 1996
PIPPIN	defective root meristem	Scheres *et al.*, 1996
ABERRANT LATERAL ROOT FORMATION4	no lateral primordia	Celenza *et al.*, 1996
SHORT ROOT	no endodermis	Benfey *et al.*, 1996; Scheres *et al.*, 1995
PINOCCHIO	no cortex	Scheres *et al.*, 1995
SCARECROW	no segregation of endodermal/cortex fates	Scheres *et al.*, 1995; Malamy and Benfey, 1997
WOODEN LEG	too few vascular cells	Scheres *et al.*, 1995
GOLLUM	atypical vascular organisation	Scheres *et al.*, 1995
TRANSPARANT TESTA GLABRA	ectopic root hairs	Galway *et al.*, 1994
GLABRA2	ectopic root hairs	Masucci *et al.*, 1996
CONSTITUTIVE TRIPLE RESPONS1	ectopic root hairs, short root//defective	Kieber *et al.*, 1993
ECTOPIC ROOT HAIR1-3 (ERH2=POM1)	downegulation of ethylene	Schneider *et al.*, 1997
ROOT HAIRLESS1-3	root hairless	Schneider *et al.*, 1997
Meristem activity		
ROOT MERISTEMLESS1,2	no post-embryonic root meristem activity	Cheng *et al.*, 1995
STUMP	no post-embryonic root meristem activity	Berleth *et al.*, 1996
ABERRANT LATERAL ROOT FORMATION3	no continued root meristem activity	Celenza *et al.*, 1995
CYC1AT		Hemerly *et al.*, 1992
CDC2a		Ferreira *et al.*, 1991
CDC2b		Imajuku *et al.*, 1992
SUPERROOT	elevated auxin levels	Boerjan *et al.*, 1995
Cell expansion		
ROOT HAIR DEFECTIVE3	no cell expansion	Schiefelbein and Sommerville, 1990; Wang *et al.*, 1997
DIMINUTO	no cell expansion	Takahashi *et al.*, 1995
LION'S TAIL	no cell expansion	Benfey *et al.*, 1993; Hauser *et al.*, 1995
STUNTED PLANT1	cells in elongation zone fail to elongate	Baskin *et al.*, 1995
COBRA	abnormal cell elongation	Benfey *et al.*, 1993; Hauser *et al.*, 1995
CUIL	deregulated expansion, epidermal	Hauser *et al.*, 1995
CUDGE	deregulated expansion, epidermal	Hauser *et al.*, 1995
POM-POM1	deregulated expansion	Hauser *et al.*, 1995
POM-POM2	deregulated expansion	Hauser *et al.*, 1995
SABRE	abnormal cortical cell expansion	Benfey *et al.*, 1993; Aeschbacher *et al.*, 1995; Hauser *et al.*, 1995
EPIDERMAL BLEBBING	blebbing of outer epidermal cell wall	Baskin *et al.*, 1992
RADIALLY SWOLLEN1-3	fat roots	Baskin *et al.*, 1992
ROOT HAIR DEFECTIVE6	defective root hair initiation	Masucci and Schiefelbein, 1994
ROOT HAIR DEFECTIVE1,2,4	defective root hair enlargement	Schiefelbein and Sommerville, 1990
TIP1	abnormal root hair tip growth	Schiefelbein *et al.*, 1993
LONG HYPOCOTYL3	long root hairs when grown in light	Reed *et al.*, 1993
Response to the environment		
WAV1	no root tip rotation	Okada and Shimura, 1990
WAV2,3	high rate of root tip rotation	Okada and Shimura, 1990
WAV4	irregular timing of reversion of root tip rotation	Okada and Shimura, 1990

Table 1. Arabidopsis *genes and the phenotype they display in roots.*

Gene	Phenotype	References
REDUCED ROOT GRAVITOPISM1	reduced response to gravity	Simmons *et al.*, 1995
AGR1=WAV6	reduced response to gravity	Bell and Maher, 1990; Okada and Shimura, 1990
AUX1=WAV5	no response to gravity	Maher and Martindale, 1990; Okada and Shimura, 1990
AXR4	no response to gravity	Hobbie and Estelle, 1995
SKU1,2	exaggerated right-slanting growth	Rutherford and Masson, 1996

selection primarily serves to illustrate the four categories without aiming to be complete.

Pattern formation

During the process of pattern formation, positional information is interpreted to give rise to a specific spatial arrangement of differentiated tissues/cells. Mutants with disrupted cell or tissue patterns can be used to identify genes involved in the interpretation process itself, or in the resulting establishment of cell or tissue identity. Several recessive mutants which are defective in patterning of the *Arabidopsis* root have been identified (*tab.* 1). Here, we will restrict discussion to those that are characterized in some detail.

Two genes have been identified which are essential for the embryonic specification of the root primordium. Seedlings homozygous for mutant alleles of the *MONOPTEROS (MP)* gene lack both the root and the hypocotyl (Berleth and Jürgens, 1993). Comparison of the embryonic and post-embryonic phenotypes led to the suggestion that the *MP* gene may be primarily required for specific aspects of vascular cell development (Przemeck *et al.*, 1996). The *MP* gene has been cloned and its expression pattern is consistent with this hypothesis (Hardtke, C. and Berleth, T., personal communication). The dramatic effect of *mp* mutants on root and hypocotyl development during embryogenesis remains a strong indication for the importance of interactions between the central and basal embryo domains for proper pattern formation (Berleth and Jürgens, 1993; *fig.* 2).

hobbit Mutants lack the specification of both parts of the root cap, have no recognizable quiescent centre, and lack root meristem activity (Scheres *et al.*, 1996). The earliest reported phenotype in *hobbit* mutants is aberrant development of the hypophyseal cell region. At later stages of embryogenesis, the adjoining region of the root is not correctly specified. Therefore, the *HOBBIT* gene appears to be primarily required for the specification of the basal domain (*fig.* 2), which in turn is required for the specification of the adjoining root meristem region.

In the radial dimension, several mutants with altered tissue layers have been identified (*tab.* 1). The mutants all display an embryo phenotype, corroborating the notion that the radial tissue pattern in the seedling is set up during embryogenesis (Scheres *et al.*, 1995). The *shortroot* mutant lacks the endodermal cell layer and the second cortical cell layer in the hypocotyl (Benfey *et al.*, 1993; Scheres *et al.*, 1995). The *SHORTROOT* gene therefore appears to be necessary for controlling the number of cell layers in the cortical tissue as well as for endodermal cell fate. *scarecrow* Mutants display a very interesting and uncommon phenotype: one layer of ground meristem is missing and the remaining cell layer in the root has features of both cortex and endodermis (Scheres *et al.*, 1995; Di Laurenzio *et al.*, 1996). It has been concluded that the *SCARECROW* gene, which encodes a putative transcription factor, is involved in the execution of specific asymmetric cell divisions (Di Laurenzio *et al.*, 1996).

The circumferential pattern of alternating hair-bearing (trichoblast) and hairless (atrichoblast) epidermal cells is a conspicuous trait that has allowed the identification of many mutants (*tab.* 1). The *transparant testa glabra (ttg)* and *glabra2 (gl2)* mutants produce ectopic root hairs. Therefore the atrichoblast cell fate requires the *TTG* and *GL2* genes, which are also necessary for shoot epidermal patterning (Galway *et al.*, 1994; Masucci *et al.*, 1996). In the latter case they promote hair-bearing (trichome) epidermal cell fate. The *GL2* gene contains a homeodomain sequence (Rerie *et al.*, 1994) and *ttg* mutants can be complemented by the maize transcription factor *R* (Lloyd *et al.*, 1992). In addition to the emerging transcription factor module for atrichoblast specification, the *ROOT HAIRLESS* genes may be involved in specification of trichoblast cell fate (Schneider *et al.*, 1997). Genetic and experimental studies on epidermal cell patterning have revealed roles for the phytohormones auxin and ethylene in root hair cell specification (*tab.* 1; Dolan *et al.*,

1994; Masucci and Schiefelbein, 1994, 1996; Tanimoto *et al.*, 1995). The role of ethylene in the initiation of root hair formation may be permissive, but the differential sensitivity to ethylene of epidermal cell files in *ttg* and *gl2* mutants points to an additional, instructive role for the ethylene signaling pathway in patterning the epidermis (Cao, X. F. and Dolan, L., personal communication).

The majority of patterning mutants show the same phenotype in the primary root formed in the embryo, and in secondary roots that are formed post-embryonically (Scheres *et al.*, 1995; 1996). This implies that many embryonic patterning genes are recruited again during secondary root formation.

Meristem activity

The ability to undergo continuous cell division is one of the defining characteristics of the root meristem. As discussed above, a mitotically active root meristem is absent in seedlings homozygous for mutant alleles of the *MONOPTEROS* and *HOBBIT* genes. However, these genes are required early, and they presumably affect cell division in the root meristem indirectly via a chain of intermediate events. Mutants with more specific defects are required for genetic analysis of mitotic control in the root meristem. The exact knowledge of cell pattern and cell numbers in the mature embryo have allowed the selection of mutants with a correctly patterned root apex, but with defects in the onset of post-embryonic divisions (*tab.* 1; Cheng *et al.*, 1995; Berleth *et al.*, 1996; our unpublished data). The identified mutants may pinpoint genes that are specifically involved in triggering and maintaining cell divisions in the meristem, although it still remains possible that physiological defects cause the mutant phenotypes.

Control of the onset and maintenance of the cell cycle is also relevant for the formation of secondary roots. New lateral root primordia originate from cells in the pericycle layer, which become mitotically activated after a period of cell cycle arrest. Classical studies have demonstrated a link between the phytohormone auxin and lateral root formation (Torrey, 1950). Lateral root mutants have confirmed this link to auxin, and these mutants may serve as an entrance for studying the mechanisms involved in lateral root formation (Boerjan *et al.*, 1995; Celenza *et al.*, 1995).

Cell division in *Arabidopsis* has also been studied by the isolation of genes homologous to two yeast cell cycle regulator families, the cyclin-dependent kinases and the cyclins (Ferreira *et al.*, 1991; Hemerly *et al.*, 1992; Imajuku *et al.*, 1992). Expression of the *CDC2a* gene marks cells which are competent for cell division (Hemerly *et al.*, 1993), and overexpression accelerates cell division. Expression of a dominant negative CDC2a was lethal in *Arabidopsis* and inhibited cell division rate in tobacco (Hemerly *et al.*, 1995). Interestingly, these tobacco plants had larger cells but no altered cell morphology. In contrast, overexpression of the *CYC1AT* gene has been reported to lead to an increase in root growth (Doerner *et al.*, 1996). More research is needed to explain these apparently contradictory effects of cell cycle gene expression on root growth.

Cell expansion

Plant cell expansion involves cell wall changes which allow osmotically based turgor pressure to drive cell enlargement (Cosgrove, 1993). The direction and rate of cell expansion is a major factor in morphogenesis as it determines not only the shape of the cell but also that of the organism (whether or not feedback mechanisms occur at the organism level). Despite many studies on the role of phytohormones, cytoskeletal proteins and cell wall loosening enzymes, the molecular mechanisms underlying cell elongation are not well understood. The *Arabidopsis* root undergoes well-described expansion in the elongation zone as well as during root hair formation, which has allowed genetic studies on cell expansion. Mutants with deregulated cell expansion representing many different genes have been identified (Schiefelbein and Somerville, 1990; Baskin *et al.*, 1992; Benfey *et al.*, 1993; Hauser *et al.*, 1995). Together, these mutants suggest complex, often tissue-specific controls on cell elongation. In the case of *sabre* mutants, interactions with phytohormones were observed and it was proposed that the *SABRE* gene product counteracted ethylene-dependent radial cell expansion (Aeschbacher *et al.*, 1995). It is of interest that root expansion mutants can be either defective in the extent or the orientation of expansion or in both (Hauser *et al.*, 1995), which implies that cell expansion is under extensive genetic regulation. In many cases, the effect on cell elongation of the mutants is not restricted to the root. For example, screens for hypocotyl elongation mutants have identified the *procuste* mutants, which turned out to be allelic to the *cuil* root expansion mutant (Höfte, H., personal communication). In conclusion, root expansion mutants provide entrances to the regulation of cell

morphology at multiple levels, and the identified genes may act either in a global or organ- and tissue-specific manner. Integration of genetic and physiological approaches may help to unravel the complex control of cell expansion as a necessary step towards the understanding of plant morphogenesis.

It is clear that cell expansion and the role of the cell wall does not relate directly to well-studied examples of animal development. Nevertheless, the identification of genes involved in this process may provide links to other organisms, aiding to the understanding of gene function. An example is provided by the recent molecular characterization of the *ROOT HAIR DEFECTIVE3 (RHD3)* gene, which shared homology with a number of genes in unrelated organisms (Wang *et al.*, 1997). In our view, an advantage in studying cell expansion of *Arabidopsis* will be the possibility to link cell-specific factors involved in the determination of cell shape to the genes that specify their expression during pattern formation.

Response to the environment

An extremely important aspect of plant development is its capacity to integrate and respond to information from the environment. Since some of the agents capable of modulating these responses, such as phytohormones, have been long known, this aspect has received considerable attention. The first clues are now being obtained on how phytohormones can combine with genetic programs to orchestrate developmental plasticity. Genetic analysis of environmental responses has been used particularly successful in the fields of light-perception and control of flowering time, but has also been applied in the root field. Roots orient their growth by responding to a number of environmental stimuli (Okada and Shimura, 1992). Due to the ease with which the orientation of root growth can be monitored, the perception of gravity, light and touch stimuli in *Arabidopsis* roots has been amenable to genetic analysis (*tab.* 1). Gravitropic mutants have been isolated using response assays and by screening for mutants with impaired sensitivity to exogenously applied auxin (Masson, 1995; Estelle, 1996). The *AUX1* gene, of which mutant alleles have been identified in both types of screens, has recently been cloned and the encoded protein is similar to amino acid transporters (Bennett *et al.*, 1996). This suggests that *AUX1* may act as an auxin transporter, which substantiates a long-proposed link between auxin transport and gravitropism. A screen based on the waving patterns of roots on tilted agar plates resulted in the isolation of mutants with altered response to gravity, to a lateral light source and to gravity-induced touch (Okada and Shimura, 1992). It is of considerable interest that the different mutants apparently define genes involved in responses specific to one stimulus, as well as genes involved in response to several of the stimuli. This implies common as well as specific branches of the signal transduction pathways involved, and hints to a complexity that cannot yet be fully grasped.

It is interesting to note that the response of plant development to the environment can be likened to animal behavior. Consequently, substantial analogies exist between genetic screens utilizing behavior to isolate developmental mutants in animals, such as screens to identify photoreceptor cell identity mutants in the fruit fly, and gravitropic mutant screens in roots. Rather then providing detailed experimental guiding, such a comparison may serve to trigger ideas which need to be worked out in a species-specific context.

Concluding remarks

The uncomplicated structure and the largely invariant cell lineage of the *Arabidopsis* root have enabled both experimental manipulation and the identification of genes involved in two aspects of root development: pattern formation and cell cycle regulation. It is noteworthy that the advantages of the root system have been exploited by methods analogous to those used in animal models, like the worm *C. elegans*. As a result, it is now evident that cells in the *Arabidopsis* root meristem are confronted with positional information. The utilization of positional information appears to be a common theme in plant development. Genes identified so far that are involved in reading out or translating positional information are first active during embryogenesis, but they are also involved in patterning lateral roots. Furthermore, both genetic and molecular approaches are providing the first insights into the regulation of the cell cycle during root development. It can be foreseen that molecular landmarks will greatly enhance the possibility to identify genes involved in these processes.

Since the shape of a plant cell is intimately linked to its cell wall, the *C. elegans* analogy is less useful when the morphogenetic aspect of root development is taken into consideration. Nevertheless, the identification of many cell expansion mutants hint that the complex

processes of morphogenesis at the cellular and organism levels are now amenable to genetic analysis.

Last but not least, *Arabidopsis* roots have proven to be powerful tools to identify genes involved in the response to various environmental stimuli. Here, parallels can be noted between plant and animal research on a completely different level, the design of "behavioral screens".

The challenge that lies ahead now is to understand the genetic programmes that determine cell fate, cell shape and cell division status in a single organ, and how these programmes interact. A second step is to combine these insights with knowledge on how these programmes can be modulated by various stimuli. It is only then that we may hope to truly understand how plant organs develop with such remarkable plasticity.

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