Root Development

Authors: Scheres, Ben, Benfey, Philip, and Dolan, Liam

Source: The Arabidopsis Book, 2002(1)

Published By: The American Society of Plant Biologists

URL: https://doi.org/10.1199/tab.0101

First published on September 30, 2002: e0101. doi: 10.1199/tab.0101

Root Development

Ben Scheres[1], Philip Benfey[2] and Liam Dolan[3]

The Arabidopsis root has a simple structure

From a developmental perspective, the Arabidopsis root is a paragon of simplicity. A small number of stem cells at the tip of the root generate all of the cell types through stereotyped divisions followed by cell differentiation and regulated cell expansion (Fig. 1a). Because root growth is indeterminate, these processes are continual, resulting in all developmental stages being present at all times. The radial symmetry of the root combined with a lack of cell movement means that clonally related cells are frequently found in cell files. These cell files can be traced back to their origins, which are four types of stem cells (or initial cells) at the root tip (Dolan et al., 1993). The epidermal/lateral root cap initials give rise to the epidermis and the outer portion of the root cap known as the lateral root cap (Fig. 1a). The central portion of the root cap, the columella, has its own set of initials. The ground tissue cells, the cortex and endodermis, are generated by division of the cortex/endodermal initials. Finally, the vascular tissue and pericycle have their own initials. Internal to and contacting all the initials is a small number of central cells that are mitotically inactive and are known as the quiescent center (QC) (Fig. 1a).

Division of initials can be either solely anticlinal (orthogonal to the axis of growth) resulting in a single file of cells or first anticlinal then periclinal (parallel to the axis of growth) resulting in two or more cell layers. The columella initials generally divide only anticlinally and their progeny undergo rapid cell expansion and then differentiate, producing starch-containing amyloplasts that play a role in gravity sensing. The other three types of initials generally undergo both anticlinal and periclinal divisions, resulting in cell lineages that acquire different identities (Dolan et al., 1993). For example the cortex/endodermal initials divide first anticlinally to regenerate the initial cell and a basal daughter cell. The basal daughter then undergoes a periclinal division to form the first cells of the cortex and endodermal lineages (Fig. 1b). These cells will undergo a small number of anticlinal divisions during which time they will acquire their specific fates. Subsequently they will undergo rapid polar expansion.

Embryonic origin of the Arabidopsis root

During Arabidopsis embryo development, cell division occurs in stereotyped patterns. This facilitates the identification of founder cells for the primary root. The origin of the quiescent centre and the columella root cap can be traced back to a single cell, the hypophysis (Scheres et al, 1994). This cell in turn derives from the basal daughter cell of the first zygotic division (Fig. 2), and is the only contribution of the basal cell to the embryo proper.

The remaining cells that will form the root in the mature embryo derive from the apical daughter cell of the zygote. Their radial organization in protodermal, ground tissue- and procambial layers is very similar to that of the entire embryo axis, except in the ground tissue (see below). Indeed, mutations in several genes that affect the radial organization of the root display identical root and hypocotyl phenotypes (Scheres et al, 1995).

The boundary between root and hypocotyl is not evident from the anatomy of the embryo although it is clearly marked post-embryonically by differentiation characteristics of individual cell types such as root hair formation and by the chlorophyll content of ground tissue cells. The hypocotyl contains three ground tissue layers whereas the majority of the root contains two. The disappearance of one ground tissue layer is visible in the embryo, but it does not coincide with the root-hypocotyl boundary as defined by cell differentiation landmarks.

Clonal analysis to analyze embryonic root development

Clonal analysis is a technique that can be used to trace the fates of the descendents of individual cells in a multicellular organism. It has been used to determine the fates of cells in the embryo that give rise to the primary root and the fate of cells in the root meristem of the seedling. Clonal analysis depends on being able to mark a cell with a visible genetic marker that can be subsequently scored. The genetic marker is inherited by all descendants of the orig-

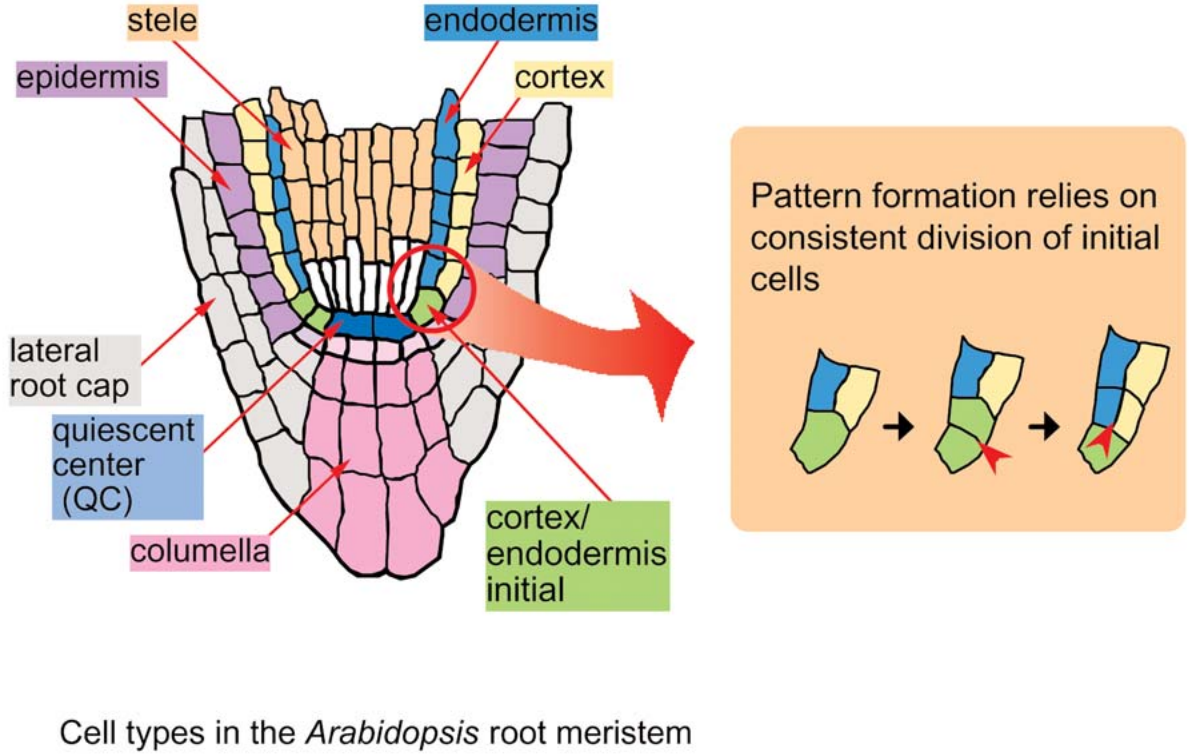


Figure 1. a) Longitudinal section of the Arabidopsis root tip that has been color-coded to show the different cell types. b) Schematic showing the two asymmetric cell divisions that the cortex/endodermis initial and its daughter undergo.

inal marked cell, and this group of marked cells constitutes a clone. The clone dimensions represent the contribution of the progenitor cell to the mature organism. Parameters such as clone size and distribution allow parameters such as cell division rates, founder cell number etc. of development to be determined. Genetic markers that have been used extensively in clonal analysis in shoots include mutations conferring chlorophyll deficiency (albino mutant) phenotypes or anthocyanin mutations that confer either intense pigment colour or loss of pigment colour. In roots there are few such markers. Instead clones have been marked with the *uid A* gene (glucuronidase gene) that catalyses the formation of a blue precipitate in cells expressing the gene when incubated with the proper substrate. In the studies described, plants were constructed that were transformed with an *uid A* gene into which an Ac or Ds transposon was inserted. Excision of the transposon from the *uid A* gene results in restoration of *uidA* activity which marks the cells.

The position of the root-hypocotyl boundary in the developing embryo has been mapped using clonal analysis (Scheres et al, 1994). Large marked clones were sampled from transgenic plants carrying such a construct, and the boundaries of these clones were analysed. For example, an excision in an embryo cell that spans all of the future hypocotyl will give rise to a hypocotyl sector (Fig. 3A). The bulk of the sector boundaries either coincided or were closely associated with embryonic cell divisions. This allowed the construction of a map that depicts the probable fate of embryonic cells, a “fate map” (Fig. 2).

Separation between root and hypocotyl domains coincided with divisions at the early heart stage of embryogenesis, but sectors marking these divisions sometimes crossed the root/hypocotyl boundary, indicating that they do not separate root and hypocotyl fates. Thus, although it is possible to map the root domain in the heart stage embryo, individual cells can respond to as yet unidentified positional cues and take on either root or hypocotyl fates until the last embryonic cell division.

The analysis also indicated that the radial arrangement

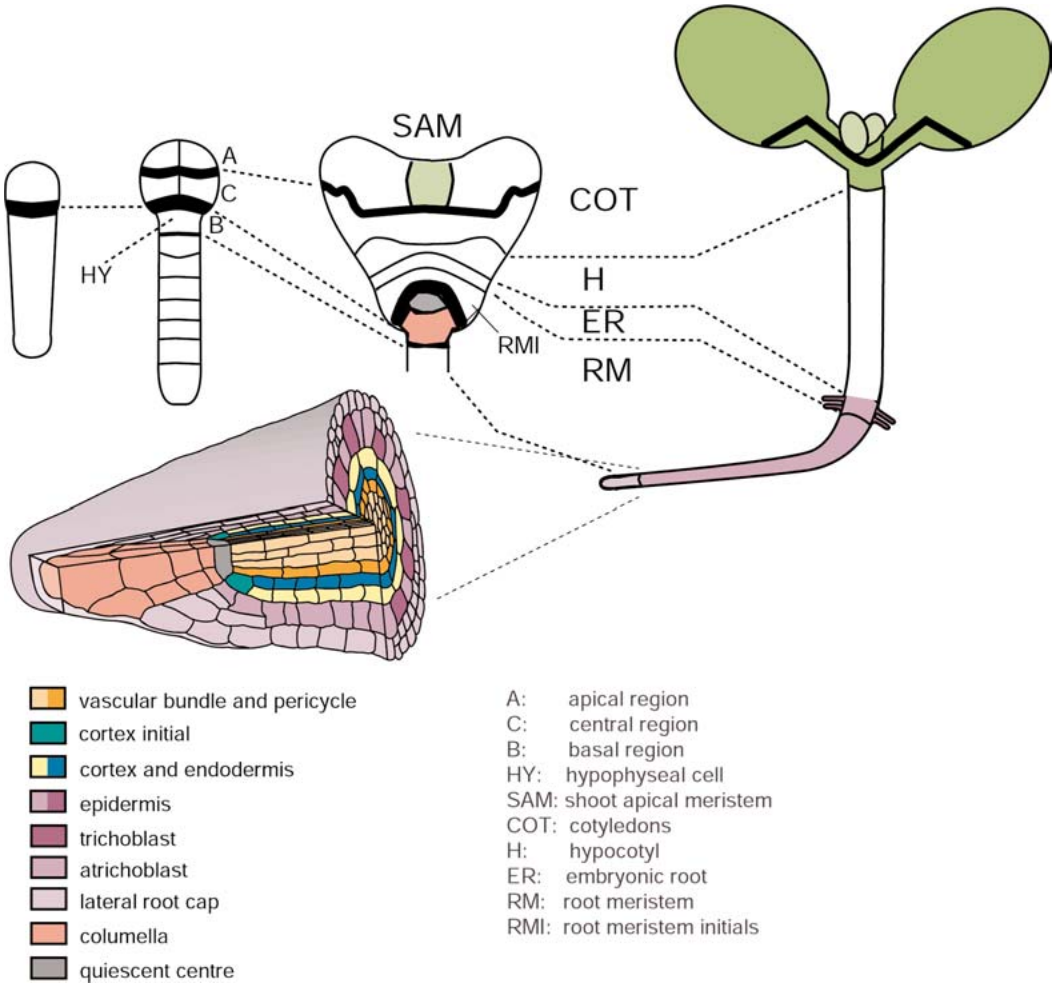


Figure 2. Embryonic origin of the *Arabidopsis* root. From left to right: first zygotic division; octant stage embryo; heart stage embryo; seedling with enlarged root meristem region.

of cells in the root is set up in the heart stage and maintained thereafter. The arrangement of initial cells around the quiescent centre, which is maintained in the mature root has been called the promeristem (Clowes, 1954). The lineage of the hypophysis is regular with no detectable variation. The marked derivatives of the hypophysis always give rise to the central cells and the columella and no sectors were observed that included the columella/central cells and other cell types of the root.

An excision in the part of the embryonic root that is outside the meristem, will give rise to an “embryonic root” sector (Fig. 3B). The root cells in this region are not surrounded by a lateral root cap layer and do not re-initiate cell division post-embryonically. They form the small junction region between root and hypocotyl with typical epidermal morphology and early emerging root hairs, the “collet”.

At the late heart stage of embryogenesis, the basalmost epidermal cell has generated a layer of lateral root cap by periclinal divisions. Anatomical and clonal analyses show that the typical stem cell-like division pattern of the root meristem originates at that stage. The cells within the developing root meristem surrounded by this first lateral root cap layer will re-initiate cell divisions after seed dor-

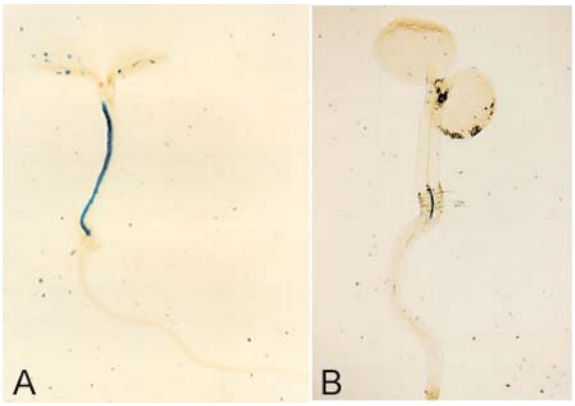


Figure 3A.. A large clone of β-glucuronidase expressing cells, originating from a single progenitor, spans the entire hypocotyl.

Figure 3B. A cell clone spanning the embryonic region of the root is marked by β-glucuronidase expression.

mancy and thus form the post-embryonic root meristem (Fig. 4).

Collectively, clonal analysis of the embryo indicates that the regulation of precise patterns of cell divisions is not required for the development of the seedling with its highly organised structure. It suggests that cell fate decisions are made independent of the lineage history of the cells involved. The absence of a role for cell lineage in the specification of cellular identity suggests that positional information is an important factor in specifying cellular identity.

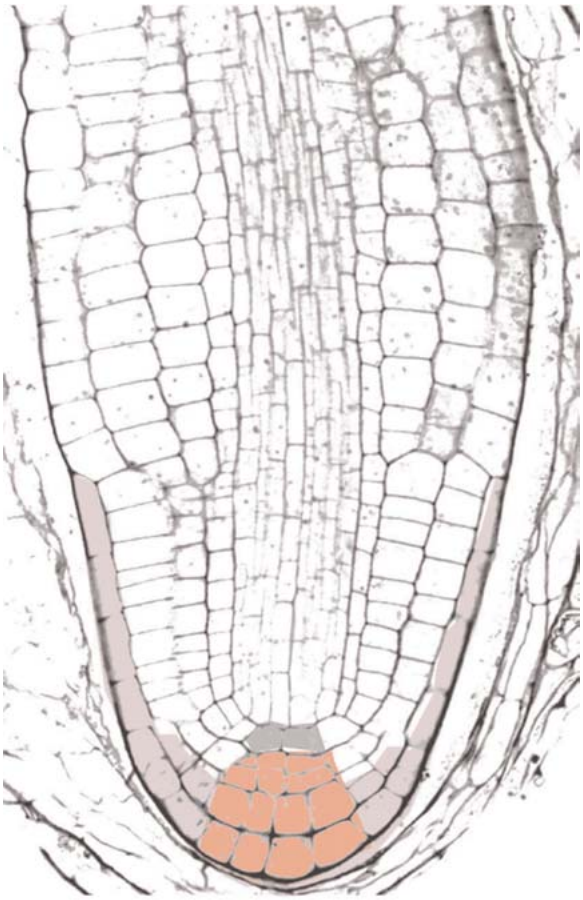


Figure 4. Lateral root cap (purple), columella root cap (pink) and quiescent center (grey) in the mature embryo. The lateral root cap layer surrounds the cells that will become mitotically reactivated upon germination to give rise to the root meristem.

Lateral root formation: a partial recapitulation of embryogenesis?

Lateral root primordia arise from pericycle cells opposite xylem poles at some distance from the primary root meristem (Dolan et al, 1993). The initial cell division patterns that give rise to new primordia are very different from those occurring during primary root formation (Casimiro et al, 2001). At later stages of lateral root formation the cellular organization becomes very similar to that of the primary root, although lateral roots display more variability in cell numbers and precise cellular organization. Analysis of cell division patterns aided by enhancer traps that mark various root tissues have been used to stage lateral root development (Malamy and Benfey, 1997). Eight stages were defined based on specific anatomical characteristics and cell divisions. Expression from cell-type specific marker lines was used to identify the cell types in the developing primordium. Analysis of the expression patterns and cell division patterns revealed that a remarkable amount of organization and cell differentiation occur at very early stages of lateral root primordium development, with differential gene expression apparent after the first set of divisions of the pericycle. In contrast, the lateral root meristem does not become active until after the primordium emerges from the parent root, and therefore does not appear to play a role in early pattern formation and organization (Malamy and Benfey, 1997). This is similar to what is seen during embryogenesis.

Cell fate studies in the post-embryonic root

Clonal analysis

The promeristem is organised during embryogenesis. Upon germination, cells of the meristem begin to divide and the root expands axially as a result of cell expansion. As the root continues to grow, the number of cells in the meristem increases and the rate of cell production increases. This increase in the number of cells can account for the doubling in the rate of root elongation between 6 and 10 days after germination. Clonal analysis has been used to investigate the fates of cells in different parts of the meristem during root growth in the young seedling (Kidner et al., 2000). The fates of central ("quiescent center") cells and initials were examined by inducing clones in 3 day old meristems using a heat inducible transposon-based marker. By subjecting roots to a short heat shock at 3 days, transposon excision from the marker gene in a small number of cells could be induced and their fate subsequently examined. Because central cells divide rarely, few sectors arising from excision events in these cells were identified. Those that were seen indicated that division of the central cell could give rise to cells in almost any cell type of the root – sectors that included the central cells also contained cells in various different tissues. This indicates that the direction of central cell division is variable. Furthermore some short sectors were found that included cells in a number of layers in the radial dimension indicating that division of some cells could give rise to cells in different layers. This indicates that while the pattern of cell division in the root is stereotypical, variations in the general pattern do occur.

Visual examination of unmarked clones has also been used to show that positional information regulates the specification of cellular identity. This relies on the ability to trace the pattern of cell divisions in "packets" of cells that are derived from a single cell in the epidermis. In this case "genetic marking" is not necessary. Rare longitudinal divisions in trichoblast cells result in the formation of twin cell files. Generally one of these twins will be in the trichoblast position overlying two cortical cells and will develop as a trichoblast (Berger et al., 1998b). The other will be in the atrichoblast position, overlying a single cortical cell when viewed in transverse section, and assumes atrichoblast identity. If position determined cell fate then it would be expected that each of the twins would assume a fate characteristic of their position – trichoblasts would form in the trichoblast location. If on the other hand, lineage were important then both twins would develop as trichoblasts, since they were derived from a trichoblast. Since the twin cells differentiate into trichoblasts and atrichoblasts in a position dependent manner it suggests that positional signals regulate the pattern if differentiation in the epdermis.

Laser ablations

Clonal analysis first indicated that the stereotyped cell divisions within the Arabidopsis embryo and root are not essential for the maintenance of early cell fate decisions. Rather, cells remain capable of adapting their fate according to their position. In clonal analysis, especially when focusing on relatively rare divisions, the cause for division plane changes that lead to unusual clones is unknown and could formally reflect an earlier fate instability, which makes it difficult to untangle cause and effect. That is why the conclusion that positional signaling is of primary importance in the post-embryonic root meristem has been extended by another technique: the use of laser ablations that lead to the expansion and subsequent division of cells into adjacent layers (van den Berg et al, 1995; Berger et al, 1998). In the ablation analyses, the cause of cell displacement is known - it is experimentally induced - and hence there is no selection for "unstable" cells, but neighbouring cell corpses could formally change the response properties of the cells that change position. Regardless of this possible caveat, ablation and subsequent reallocation of meristematic cells along the radial axis and along the apical-basal axis resulted in appropriate cell fate changes. This again suggested that cell fates depend on both radial and apical-basal positional signalling. Thus, all available data, using approaches with different advantages and disadvantages, support the same conclusion that positional information is continuously interpreted by root meristematic cells.

What is the nature of the positional information that guides distal specification in the root tip? Upon ablation of the quiescent centre cells, provascular cells in the apical-basal axis (we use apical and basal as in the embryo and hence apical is at the proximal side of the root tip and basal is at the distal end) re-specify to form columella root cap and quiescent center cells (van den Berg et al, 1997) (Figs. 5 and 6). Root tips accumulate auxins and the accumulation of a synthetic auxin responsive promoter element displays a maximum just below the QC (Fig. 7 panel A). The distal re-specification observed after laser ablation of the QC is presaged by a new expression maximum of this promoter element. Re-specification does not occur in roots treated with low levels of polar auxin transport inhibitors or in dominant *axr3* mutants (Sabatini et al 1999), which stabilize the AXR3/IAA17 transcriptional regulator of

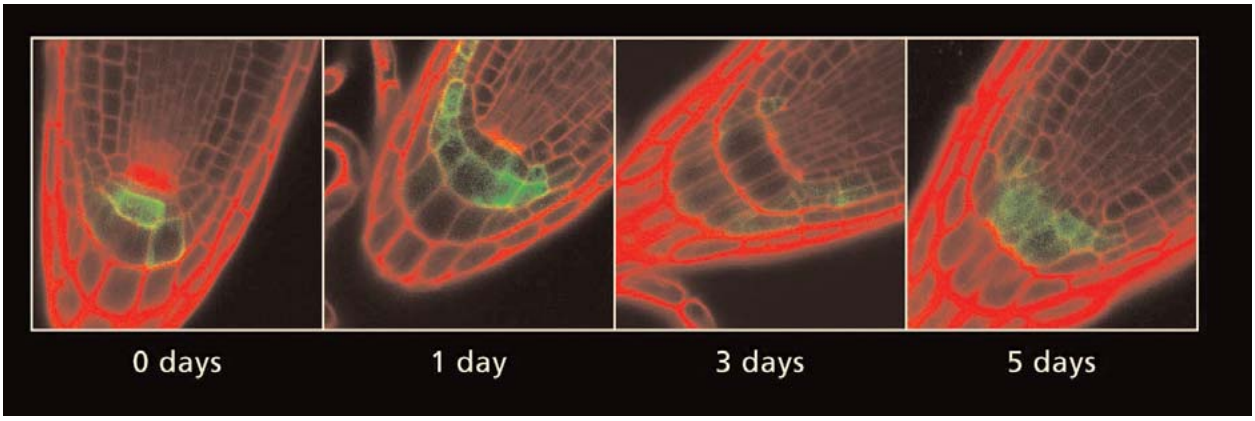


Figure 5. Laser ablation of the quiescent center in the post-embryonic root. Confocal scanning images from the same root. The cells are outlined by propidium iodide staining performed right after ablation (day 0) and prior to imaging at subsequent days. Columella cells are highlighted by the expression of GFP. At day 0, the successful ablation is marked by the entry of propidium iodine in QC cells. These cells are compressed and positioned more distally in the following days due to the differentiation and sloughing off of root cap. The columella marker shifts and becomes re-expressed in former provascular cells. After 5 days, these cells form a new columella and the cellular organization will soon be indistinguishable from untreated roots.

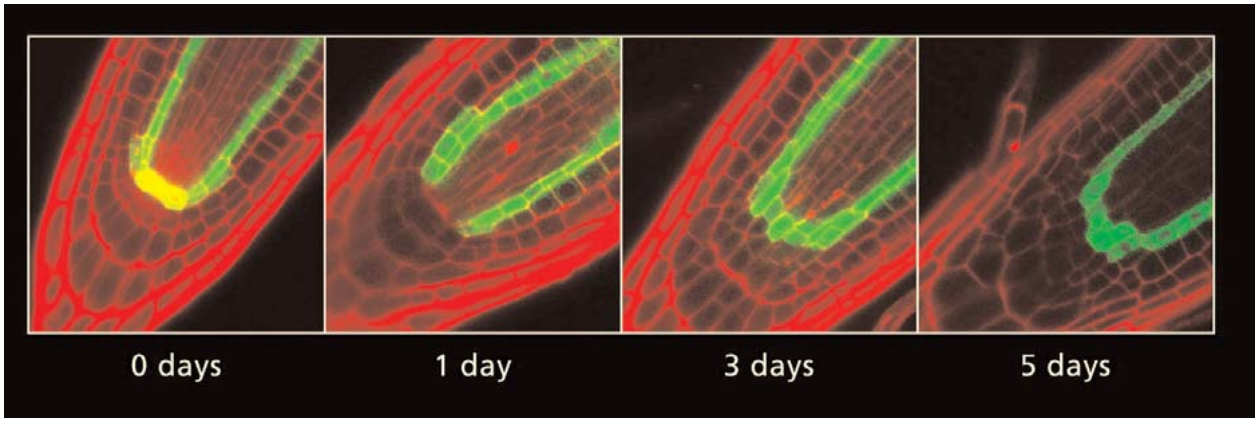
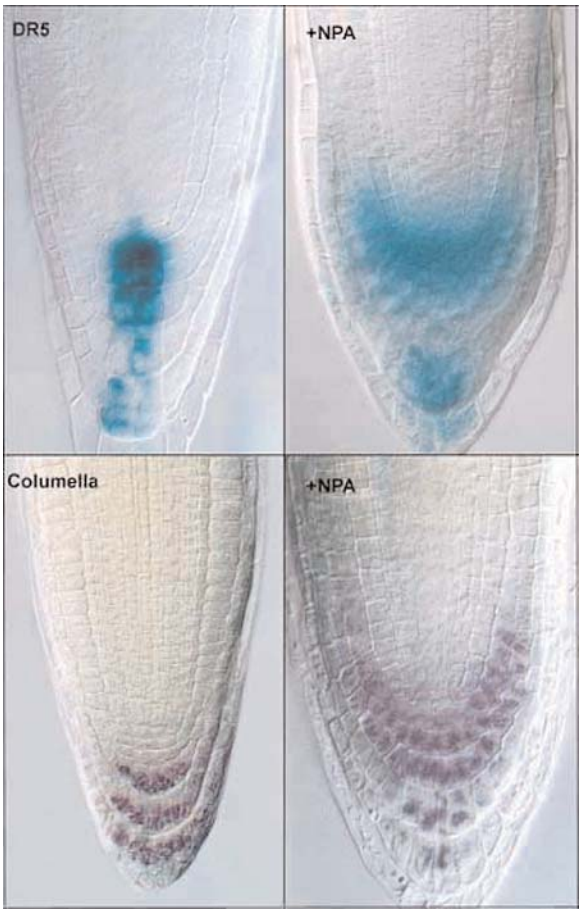


Figure 6. Laser ablation as in Fig. 5. A fusion of the *SCARECROW* promoter to GFP is used to visualize the re-appearance of quiescent center cells in the provascular domain from 3 days after ablation onward.

auxin responses (Rouse et al, 1998; Ouellet et al, 2001). Thus, accumulated auxin at the root tip is required for distal position-dependent specification. Re-localization of the auxin response pattern by treatment with high doses of polar transport inhibitors leads to the specification of QC, columella and lateral root cap layers in the entire meristem, suggesting that the perception of high auxin levels is not only necessary but also sufficient to distalize the root meristem (Fig. 7A).

An important aspect of the distal root tip is that it harbors all meristem initials, which act as stem cells for the different cell lineages. Ablation of single QC cells leads to loss of stem cell status and progression toward differentiation of the contacting initial cells (van den Berg et al, 1997). This is most clearly demonstrated by the cessation of cell division and the acquisition of starch granules by columella root cap initials. Only initials contacting the ablated QC cells are affected, suggesting that short-range signals emanating from the QC maintain stem cell activity of the surrounding initials (Fig. 7B). It is interesting to note that a similar stem cell maintenance function has been proposed for the cells underneath the stem cell layers in the shoot apical meristem (Mayer et al, 2000).

Isolation of ground tissue initial cells from more mature



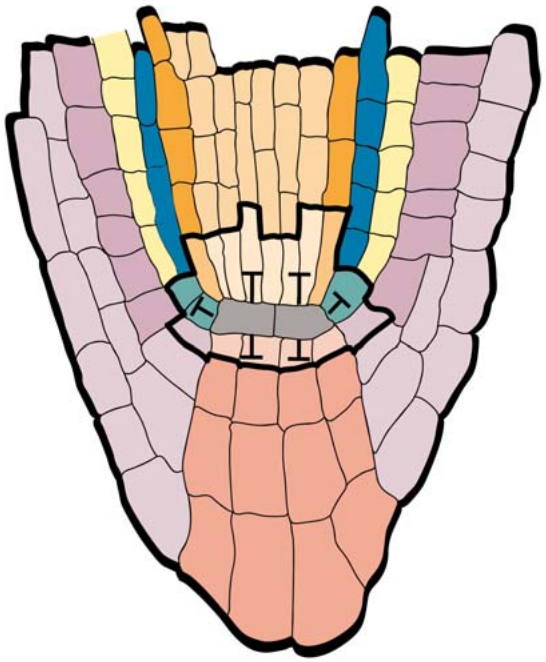


Figure 7A. The synthetic auxin response element DR5::GUS marks the distal-most cells of the root tip, and its location is changed dramatically by the addition of polar auxin transport inhibitors such as naphtylphtalamic acid (NPA). Treatment with NPA also changes the size and shape of the columella root cap domain, visualized here by their characteristic starch granules.

Figure 7B. Model for maintenance of stem cells that surround the quiescent center by short-range signals that suppress progression of cell differentiation.

ground tissue cells by ablation of their apically located daughters interferes with the change in orientation of the cell division plane required to form new endodermal and cortical cell files. Thus, cell-layer connectivity is required for an asymmetric cell division. This can be interpreted to reflect the flow of positional information from more mature cells to stem cells as a mechanism to maintain radial patterning information (van den Berg et al, 1995). However, since the QC delays differentiation or promotes stem cell fate, these observations may also reflect an accumulation of stem cell promoting factors (van den Berg et al, 1997). Ground tissue and QC ablations will have to be repeated with newly available markers for cell fate and cell differentiation status to distinguish between these possibilities.

Laser ablations have also been used to analyze the nature of cell-to-cell signalling involved in trichoblast specification (Berger et al 1998). Interestingly, cell fate was not altered when communication with living neighbour cells was impaired, leading to the suggestion that the positional cues are of extracellular nature.

Molecular Genetics of root development

Many genes have been shown to be involved in various

aspects of root development (Table 1). Below, their roles will be discussed in five major contexts: distal patterning, radial patterning, epidermal patterning, cell division and cell expansion.

Molecular genetic analysis of distal patterning

Numerous physiological experiments have demonstrated that the formation of entire root systems can be stimulated by auxins, whereas the above-mentioned experiments in *Arabidopsis* focus on auxin requirement for distal specification. The analysis of mutants impaired in root formation provides more evidence for a role for auxins in formation of the entire root as well as in the specification of its distal elements.

Mutations in several genes interfere with embryonic root formation. *monopteros (mp)* mutants lack an embryonic root altogether (Berleth and Jurgens, 1993). The *MP* gene encodes a member of the AUXIN RESPONSE FACTOR proteins that can mediate rapid transcriptional responses to auxin (Hardtke et al, 1998). The *bodenlos (bdl)* and *auxin resistant6 (axr6)* mutants show similar early embryonic phenotypes and severe primary root defects (Hamann et al, 1999; Hobbie et al, 2000). Both these mutants display enhanced resistance to externally added auxin, and the *bdl* mutant genetically interacts with *mp* mutants (Hamann et al, 1999). Thus, mutations in at least three genes point to involvement of auxins in embryonic root formation. In addition, more subtle defects in post-embryonic root structure and/or cell differentiation have been observed in *auxin resistant1 (axr1)* and *transport inhibitor resistant1 (tir1)* mutants, consistent with their proposed function in modulating auxin responses through targeted proteolysis (Leyser et al, 1993; Ruegger et al, 1998).

Mutations in the *HOBBIT (HBT)* gene also interfere with root formation. Post-embryonic meristem activity is absent and the distal cell types (QC, columella- and lateral root cap) do not differentiate (Willemsen et al, 1998). The earliest defect found in *hbt* mutants is disturbance of cell division planes in the hypophysis, the progenitor cell for the QC and columella. This defect occurs in a more limited

Table 1. Genes implicated in Arabidopsis root development

name	abbr.	required for:	encodes:	accession number (NCBI)
ABERRANT LATERAL ROOT FORMATION4	*ALF4*	lateral root formation		
AUXIN RESISTANT1	*AXR1*	root formation, auxin response	SCF activating factor	NP_172010
AUXIN RESISTANT3/IAA17	*AXR3*	auxin response (domin. allele)	AUX/IAA protein	NP_171921
AUXIN RESISTANT6	*AXR6*	root formation, auxin response		
BODENLOS	*BDL*	root formation, auxin response		
CAPRICE	*CPC*	promotes hair cell development	transcription factor	E14755
COBRA	*COB*	oriented cell expansion	GPI-anchored protein	AF319663
CUDGEL	*CUD*	radial expansion		
GLABRA 2	*GL2*	promotes non hair cell development	transcription factor	L32873
GNOM	*GN*	cell and organ polarity	ARF-GEF	S65571
HOBBIT	*HBT*	cell division, differentiation	CDC27 homologue	
KOJAK/ ATCSLD3	*KJK*	cell wall biosynthesis	transglycosylase	AF232907
LEUCINE RICH EXPANSIN1	*LRX1*	cell expansion	expansin	AY026364
LION'S TAIL/KORRIGAN	*LIT/KOR*	Radial cell expansion	endoglucanase	AF073875
MONOPTEROS	*MP*	embryo-, vascular development	transcription factor	T51807
NAC1	*NAC1*	lateral root formation	transcription factor	AAF21437
PIN-FORMED INFLORESCENCE LIKE4	*PIN4*	root formation	putative efflux carrier	AF087016
POM-POM1	*POM1*	Radial cell expansion		
POM-POM2	*POM2*	Radial cell expansion		
PROCUSTE1/QUILL/ATCESA6	*PRC1/QUI*	Radial cell expansion	Putative cellulose synthase subunit	
ROOT HAIR DEFECTIVE3	*RHD3*	required for root hair growth	G protein	U86081
ROOT HAIRLESS1	*RHL1*	required for initiation of root hairs	Nuclear protein	AF062372
RADIAL SWELLING/ATCESA1	*RSW1*	Radial cell expansion	Putative cellulose synthase subunit	AF027172
SCARECROW	*SCR*	Division of ground tissue initial	Putative transcription factor	U62798
SHORT-ROOT	*SHR*	Specification of endodermis	Putative transcription factor	AF233752
SOLITARY ROOT/IAA14	*SLR*	lateral root form. (domin.allele)	AUX/IAA protein	AAG50096
SUPERROOT	*SUR*	lateral root formation		
TINY ROOT HAIR1	*TRH1*	root hair elognation	K^+ carrier	AJ296156
TORNADO	*TRN*	epidermal cell identity		
TRANSPARENT TESTA GLABRA	*TTG*	promotes non hair cell development	WD40 repeat containing protein	AJ251522
TRANSPORT INHIBITOR RESISTANT1	*TIR1*	auxin response	F-box protein	AF327430
WEREWOLF	*WER*	promotes non hair cell development	transcription factor	AF126399
WOODEN LEG	*WOL*	Patterning in stele	Two-component kinase	AJ278528

embryo domain and also at a later stage than *mp*, *bdl* and *axr6* defects. *hbt* mutants respond to IAA, but the synthetic auxin response element DR5 can be induced only at high auxin concentrations, suggesting that transcriptional responses to auxin are desensitised in the mutant background (Blilou et al, in prep). The *HBT* gene encodes a homologue of a subunit of the yeast Anaphase Promoting Complex (Blilou et al, in prep), and may be required in the incipient root primordium to allow auxin-dependent root development and at later stages to allow auxin-dependent differentiation of distal cell types.

Components of putative efflux carriers that are required for polar transport of auxin may also display subtle defects in the embryonic root pole. For example, *pin4* mutants show aberrant divisions of the hypophyseal cell and changes in the distribution of the auxin reporter maximum in the root tip (Friml et al, 2002). Mutations in the *GNOM* gene, encoding an ARF-GEF protein involved in vesicle trafficking, disrupt the coordinate polar localization of at least one of the efflux carriers (Steinmann et al, 1999). It will be interesting to find out whether polar auxin transport defects underly the inability of *gnom* seedlings to form a root primordium.

While many genes have been identified that connect root development to auxins, transcription factors that specify the different distal cell types have not yet been reported. Several factors of the MADS box class (Alvarez-Buylla et al, 2000) and two AP2 type transcription factors (Aida et al, in prep.) are currently being investigated, and new tools of the post-genomic era may aid in the identifi-

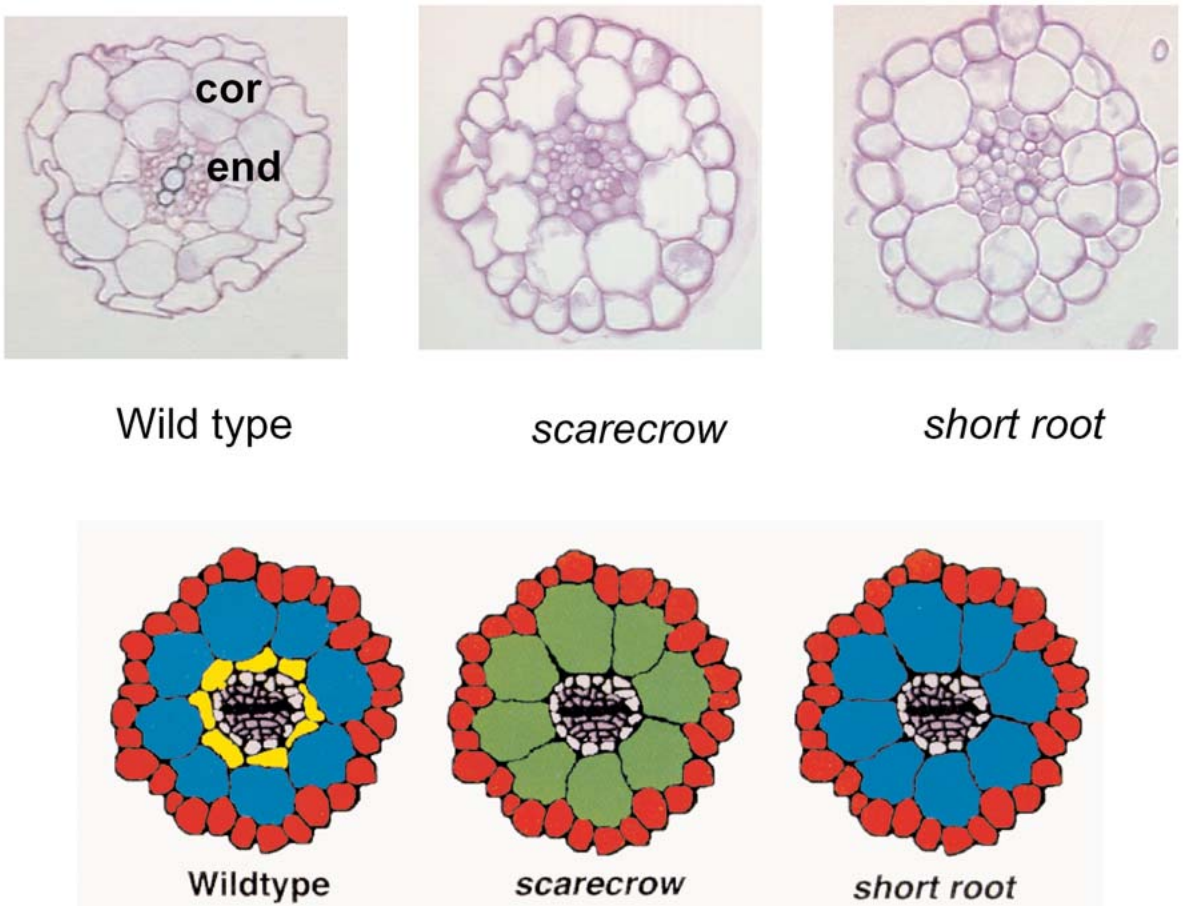


Fig. 8. Radial organization of Arabidopsis roots. The *scarecrow* mutant is missing one cell layer, and the resulting mutant layer has differentiated attributes of both cortex and endodermis. The *short root* mutant is also missing a cell layer, but its mutant layer has attributes of only cortex.

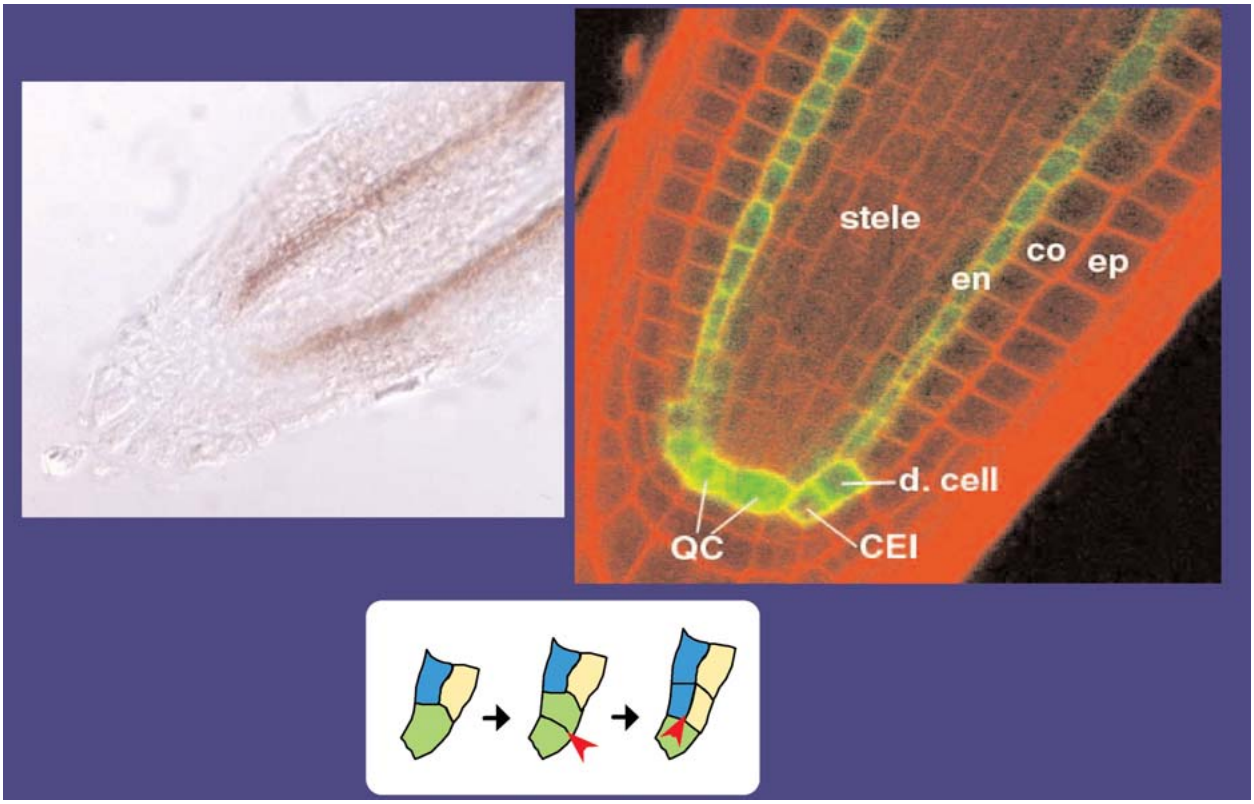


Figure 9. a) RNA expression pattern of *SCARECROW* as determined by in situ hybridization (Di Laurenzio et al., 1996). b)Expression conferred by the *SCR* promoter fused to GFP (Wysocka-Diller et al., 2000).

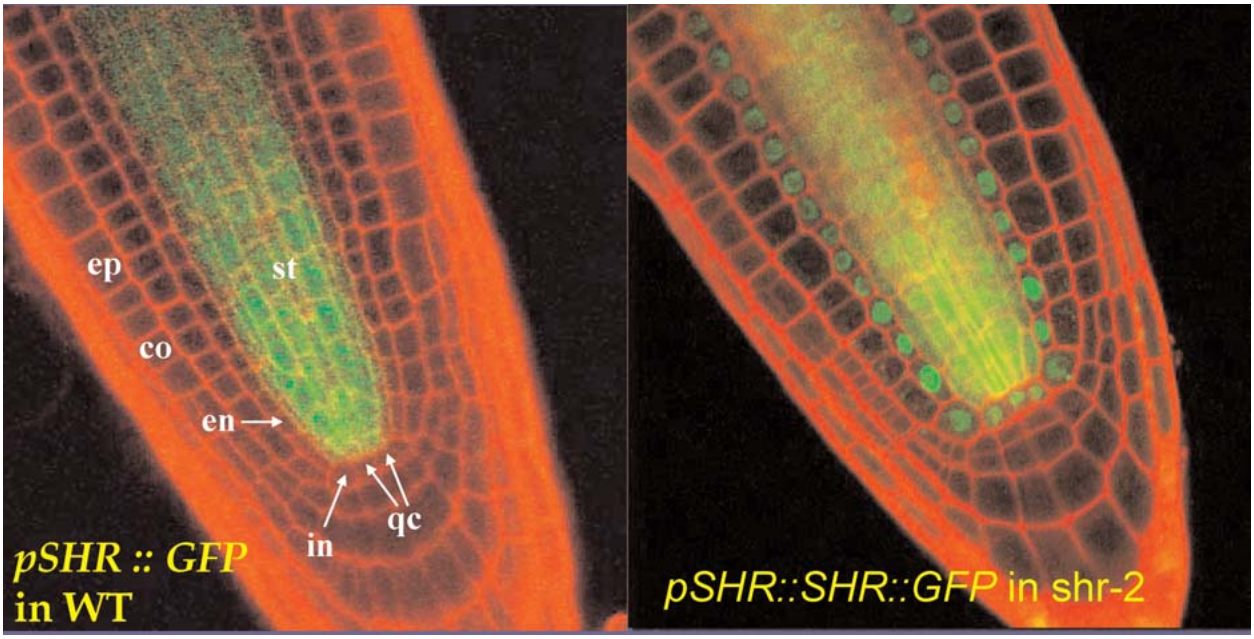


Figure 10. a) Expression conferred by the *SHR* promoter fused to GFP. B) Expression from a translational fusion of SHR to GFP, driven by the *SHR* promoter introduced into the *shr-2* mutant background (Nakajima et al., 2001).

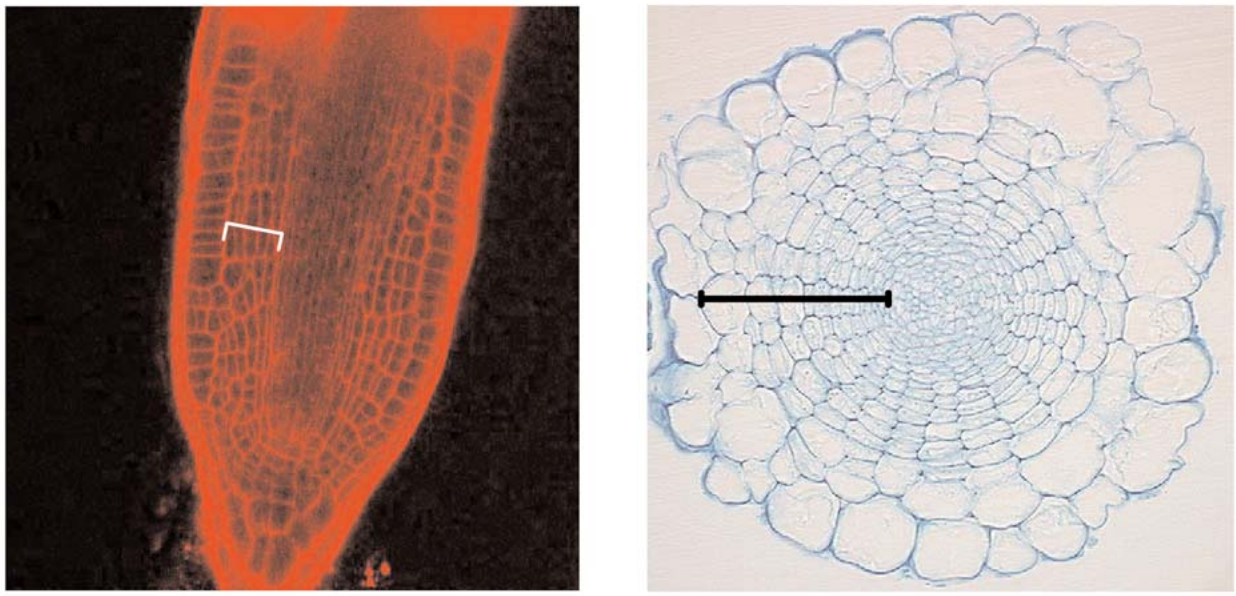


Figure 11. a) Longitudinal optical section of transgenic plants expressing SHR from the SCR promoter. Note the supernumerary ground tissue layers. b) Transverse section from roots of the same plants (Nakajima et al., 2001).

cation of other ones.

In *tornado* (*trn1* and *trn2*) mutants the formative divisions of the epidermal/lateral root cap initial are defective. Moreover, cells with lateral root cap attributes are found in the outermost cell layer, which normally contains only epidermal cells (Cnops et al., 2000). This indicates that TRN1 and TRN2 are required for correct cell specification in the outermost layer of cells and that the role of these gene products is to repress lateral root cap fate in cells in the epidermal location. The molecular nature of the TRN gene products is not yet known. The epidermal fate changes in *trn* mutants may indicate that the specification of the lateral root cap as a distal pattern element involves interaction with epidermal factors that are themselves dependent on radial patterning events.

If primary root development strongly depends on the phytohormone auxin, it seems reasonable to expect a similar important role for auxins in lateral root initiation. Indeed, genetic analysis of the formation of lateral roots has also revealed numerous links to auxins. Exogenous auxin addition leads to supernumerary roots, and *superroot* mutants (also referred to as *aberrant lateral root formation1* and *hookless3*) display a similar phenotype. (Boerjan et al, 1995, Celenza et al, 1995). Auxin-resistant mutants have a reduced number of lateral roots (Hobbie and Estelle, 1995; Timpte et al, 1995). The auxin-inducible NAC1 transcription factor, whose activity determines the number of lateral root primordia, acts downstream of *tir1*-dependent auxin responses (Xie et al, 2000). Lateral root formation is not stimulated by IAA in *aberrant lateral root formation4 (alf4)* mutants, suggesting that the corresponding gene also acts downstream of an initial IAA-related stimulus for lateral root formation (Celenza et al, 1995). Lastly, the *solitary root* mutant with a specific defect in lateral root formation carries a dominant mutation in a gene encoding an AUX-IAA protein family member, suggesting a specific role for this particular member in the induction of lateral roots (Fukaki et al, 2002). The many mutants and corresponding genes discussed now provide an entry to dissect the mechanisms by which auxins appear to promote lateral root development. It will be interesting to determine the extent of overlap and specific differences between inductive processes in lateral roots to those operating in the embryo.

Molecular genetic analysis of radial patterning

The radial organization of the root is generated by stereotyped division of initial cells and subsequent acquisition of cell fate. In a transverse root section, there are four radially symmetric layers (from outside in, epidermis, cortex,

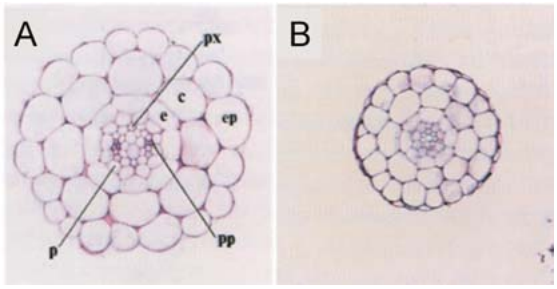


Figure 12. a) Cells in wild type vascular tissue b) Only protoxylem cells are found in the *wooden leg* vascular tissue (Scheres et al, 1995?).

endodermis, pericycle) that surround the bilaterally symmetric vascular tissue (consisting of phloem, xylem and procambium) (Scheres et al., 1995) (Fig. 8a). The vascular tissue and surrounding pericycle are termed the stele. Mutations that disrupt the radial pattern have been useful in identifying genes that play important roles in establishing and maintaining the pattern. Many of these mutations were isolated by screening for roots that were no longer able to grow in an indeterminate fashion. From these screens, mutations that disrupt patterning of the ground tissue and vascular cylinder have been identified (Scheres et al., 1995).

In both the *scarecrow* (*scr*) and *short-root* mutants, instead of cortex and endodermis there is a single mutant layer between the epidermis and stele (Scheres et al., 1995) (Fig. 8b, 8c). Analysis of tissue-specific markers revealed that the mutant layer is specified differently in the two mutants. In *scr*, markers for both cortex and endodermis are present in the mutant layer, indicating that SCR is required for the periclinal division of the initial cell, but does not play a role in cell specification (Di Laurenzio et al., 1996) (Fig. 2b). In *shr*, only markers for cortex are found, indicating that SHR is required for both the longitudinal cell division of the initial as well as endodermal cell specification (Di Laurenzio et al., 1996) (Fig. 2c). Both genes encode members of the GRAS family of putative transcriptional regulators. *SCR* is transcribed in the cortex/endodermal initial, its daughter and all of the endodermal cells as well as in the QC (Di Laurenzio et al., 1996) (Fig. 9). Because *SCR* expression is down regulated in a *shr* mutant background, it was concluded that SHR is required for full transcriptional activation of *SCR* (Helariutta et al., 2000). However, *SHR* RNA is found exclusively in the stele cells (Fig. 4a), indicating that it acts non-cell autonomously. Immunolocalization of SHR protein as well as fusions of SHR to Green Fluorescent Protein (GFP) indicated that SHR may move from the stele to the adjacent cell layer. SHR protein is found not only in the stele but also in all of the cells of the adjacent layer (Fig. 10b), including the cortex/endodermal initial, its daughter, the endodermis and the QC (Nakajima et al., 2001). These results indicate that SHR protein is able to translocate from the stele to cells of the adjacent layer. Although the precise mechanism has not yet been determined, it has been hypothesized that SHR is able to move through plasmodesmata. The movement appears to be tightly regulated, as there is no evidence of SHR protein in cells external to the adjacent layer. Moreover, in the stele, SHR is localized to both nucleus and cytoplasm, but once translocated to the endodermis it is found exclusively in the nucleus (Fig. 10b) (Nakajima et al., 2001). These results indicate that SHR acts both as an activator of cell division and inducer of cell fate as well as a positional signal.

Ectopic expression of SHR driven by the constitutive 35S promoter results in supernumerary cell layers with altered cell fates (Helariutta et al., 2000). When SHR expression is driven by the SCR promoter a very specific increase in ground tissue layers is observed (Fig. 11) (Nakajima et al., 2001). In strongly expressing lines, these ectopic ground tissue cells express endodermal markers. There is a tight correlation between the presence of SHR protein and endodermal cell fate, suggesting that SHR may be sufficient to induce endodermal cell specification. Introgressing the *scr* mutation into these lines demonstrated that the cell divisions that produce the supernumerary layers are dependent on SCR activity (Nakajima et al., 2001).

In the *wooden leg* (*wol*) mutant, protoxylem is the only tissue in the vascular cylinder (Fig. 12b, compare with wild type in Fig. 12a) (Scheres et al., 1995) (Mahonen et al., 2000). Normally phloem and procambium are established through a set of asymmetric cell divisions of their initials. These divisions require the *WOODEN LEG* (*WOL*) gene, which is expressed in the vascular tissue of the root beginning at the early stages of embryogenesis (Mahonen et al., 2000). The *WOL* gene encodes a novel two-component histidine kinase. There is evidence that the *WOL* gene product is able to bind cytokinin raising the possibility that this phytohormone plays a direct role in regulating the patterning of the vasculature (Inoue et al., 2001).

Molecular Genetic Analysis of epidermal patterning

(see chapter 75 by Schiefelbein & Grierson)

Specification of cell identity

The root epidermis is composed of two cell types whose identity is regulated by positional information. Trichoblasts develop into hair cells and are located in the cleft between underlying cortical cells when (viewed in transverse section) while atrichoblasts remain hairless and are located over single cortical cells (Dolan et al., 1994; Galway et al., 1994). Laser ablation experiments and clonal analysis indicates that positional cues direct cell identity and there is some evidence that these positional cues are located in the cell wall (Berger et al., 1998b).

Cell identity is regulated in the epidermis by a cascade of transcriptional regulators. GLABRA 2 is a positive regulator of atrichoblast/non hair cell fate and is expressed in the atrichoblast /non hair cells (Masucci and Schiefelbein). GLABRA 2 is positively regulated by WEREWOLF, a MYB-related putative transcriptional regulator that is expressed in the atrichoblasts/non hair cells, and TRANSPARENT TESTA GLABRA, a WD repeat protein that is proposed to mediate protein-protein interactions (Lee and Schiefelbein 1999; Galway et al., 1994). Trichoblast fate is positively regulated by the Myb- related protein, CAPRICE (Wada et al, 1997). It has been proposed that the balance between the two Myb related proteins determines cell fate; high WER/CPC in atrichoblasts/non hair epidermal cells and low WER/CPC in trichoblast/hair cells (Lee and Schiefelbein, 1999) (see chapter N for full discussion).

Once hair cells have been specified, the hairs are initiated from the outer side of the hair cell nearest the meristem. The polar localisation of the hair cell is dependent on an ethylene and auxin pathway and coincides with the establishment of a high Ca^{2+} gradient in the hair tip (Schiefelbein et al., 1992;Wymer et al 1997). Tip growth is established and hairs elongate. While a large number of mutants with defects in hair growth have been identified, to date few have been characterised in detail (Schiefelbein and Somerville, 1990; Schneider et al., 1997; Parker et al., 2000). ROOT HAIRLESS1 is a nuclear protein required for hair initiation (Schneider et al., 1998). ROOT HAIR DEFECTIVE3 is a small G-protein required to control the direction of tip growth (Wang et al., 1996). KOJAK/ AtCSLD3 is required for the synthesis of cell wall polysaccharide (Favery et al.,2001; Wang et al., 2001). LEUCINE RICH EXPANSIN1 (LRX1) is cell surface protein required to maintain cell shape (Baumberger et al., 2001). TINY ROOT HAIR3 is a potassium carrier required during initiation (Rigas et al., 2001). Together these molecular and genetic analyses are contributing to the construction of a model for hair cell growth.

Cell division in the root (see Chapter 28 by Doerner)

New cells are produced in the meristem, in which distinct zones of cell division activities are evident. The central "quiescent center " cells divide infrequently, and cell division rates increase progressively up the root until a maximum is reached at a point that is dependent on root age and growth conditions (Beemster and Baskin, 1998). By measuring the incorporation of the nucleotide analogue bromo deoxyuridine (BrdU) into replicating DNA, it was shown that 7% of cells incorporated the precursor into the nuclei of central cells in a 24 hour period (Fujie et al., 1993). The initial cells that surround the central cells incorporated precursor more frequently – 28% of these cells incorporate precursors. 84% of nuclei in cells located two cell distances from the central cells incorporated DNA precursors. This is consistent with the central cells having very low rates of cell divisions with increasing rates of division in cells at greater distance from the central cells. Cell division rates increase from the central cells to a point behind the tip that varies as the root ages. In 6-day old meristems a cell division peak is observed around 600μm from the central cells while in older roots the peak is further back from the tip at approximately 1000 μm.

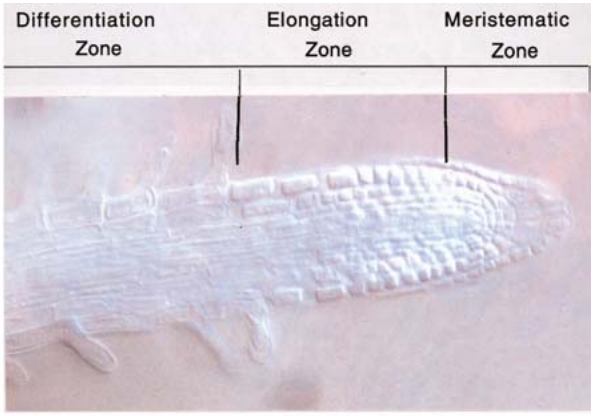


Figure 13. Cell expansion in the root. In the meristematic zone cells are generally isodiametric. As they traverse the elongation zone, radial expansion ends and rapid longitudinal expansion occurs.

Cell division in the epidermis is regulated by a subset of the genes that regulate cell identity

Trichoblasts are shorter than atrichoblasts throughout their development through the meristem, elongation and differentiation zones Dolan et al., 1994; Galway et al., 1994). The difference in cell length is visible already near the promeristem and is subsequently maintained through differentiation (Beemster and Baskin, 1998). Because the two cell types differ in length from early development through to the mature stages, the rates of cell division are identical in both cell types through the meristematic zone. Nevertheless the difference in cell size between the two cell types is being constantly regulated. While the majority of cell divisions in the epidermis are transverse, adding more cells to each file, occasional longitudinal divisions occur that result in the formation of a pair of cells side by side (twins) resulting in a file duplication (Berger et al., 1998). Both daughter cells are the same length but after a few rounds of division the cells in the trichoblast position that are derived from one of the twins becomes shorter than those derived from the other twin in the atrichoblast position. For cells in the trichoblast position to be shorter than those in the atrichoblast position the rates of cell division must be greater in the trichoblasts than atrichoblasts. Therefore upon switching positions the cell division parameters are altered, resulting in the formation of cells with the appropriate dimensions. This indicates that relative cell size and cell division rates are strictly regulated in the epidermis.

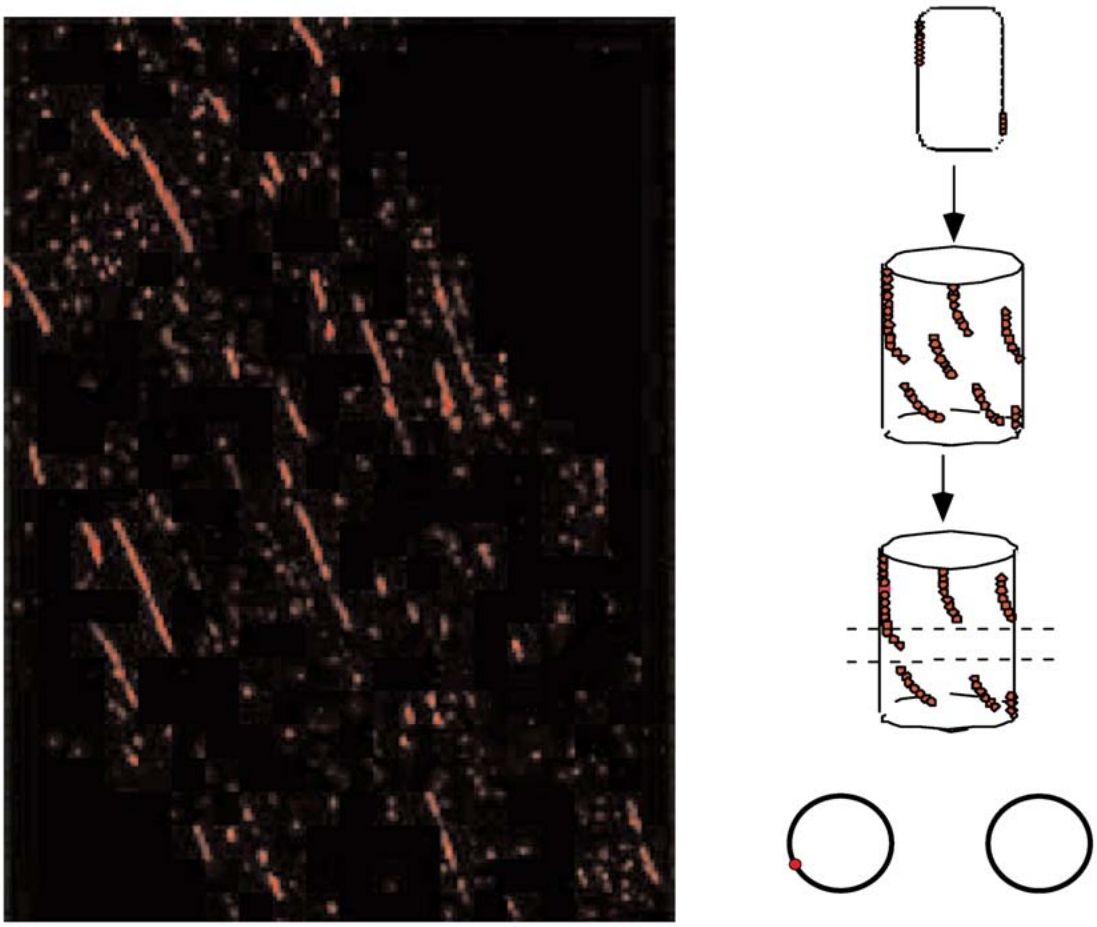


Figure 14. Antibody staining of COBRA protein in root cells. The protein appears to be preferentially localized to the longitudinal sides of the cells (Schindelman et al., 2001).

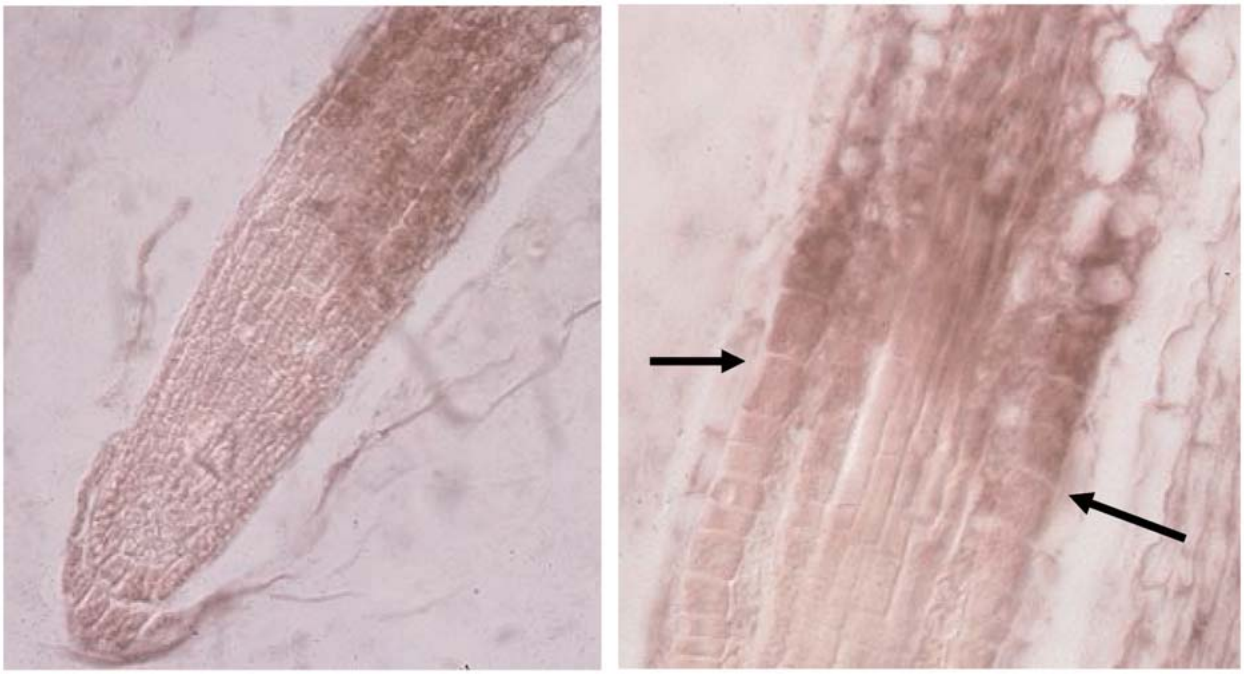


Figure 15. Expression of *COBRA* RNA as determined by *in situ* hybridization. Expression is dramatically upregulated as cells begin the phase of highly polarized longitudinal elongation (Schindelman et al., 2001).

The longitudinal divisions that produce twin cells giving rise to file duplications take place predominantly in cells in the trichoblast position. The restriction of these longitudinal divisions to the trichoblasts is controlled by a subset of the genes that regulate cell type specification in the epidermis (chapter by Schiefelbein and Grierson). *transparent testa glabra* (*ttg*) mutants develop hairs on every cell in the epidermis but also undergo longitudinal divisions in both atrichoblast and trichoblast positions, indicating that TTG is also required for the suppression of longitudinal divisions in the atrichoblasts (Berger et al., 1998). On the other hand every epidermal cell in *glabra2* (*gl2*) mutants develop hairs but no longitudinal divisions occur in the atrichoblasts [if every cell develops a hair, then where are the atrichoblasts?], indicating that the specification of cell fate and longitudinal divisions can be uncoupled. Together these data indicate that a subset of the genes regulating cell specification also regulate the plane of cell division in the epidermis.

Root cell expansion

Because there is no cell movement during plant development, cell expansion is one of the key parameters that determine the ultimate form of plant organs. How far a cell expands and in what direction are the two primary determinants of a cell's final shape. The *Arabidopsis* root provides an ideal system in which to analyse cell expansion. In particular, mutations that affect the extent or orientation of cell expansion can be readily identified. Different types of cell expansion occur in different regions of the root. To generate cell files, initial cells and their immediate progeny go through a continuous process of expansion and division. At first the expansion of these dividing root cells is relatively slow and non-polar, such that their size remains fairly constant (Fig. 13). During this time, continuing cell divisions in the meristematic zone displace these cells upward in the cell file. A transition to anisotropic expansion then occurs which, when combined with cell division, results in cells with longer radial dimensions than longitudinal. This is particularly apparent in the epidermal cells (Fig. 13). During this phase the ultimate root radius is established. A second transition to highly polarized longitudinal expansion occurs in the middle of the elongation zone. By this time cell division has almost stopped. This expansion is rapid and highly oriented in the longitudinal axis.

All evidence indicates that the plant cell wall regulates both the extent and orientation of cell expansion. The primary load-bearing molecule in the cell wall is thought to be

cellulose microfibrils, which are arranged like hoops around a barrel. Thus, it is not surprising that many of the genes identified in mutant screens for altered root cell expansion appear to play a role in cellulose synthesis. For example, *rsw1* is a temperature-sensitive mutation that at the restrictive temperature has swollen cells, reduced crystalline cellulose and a lack of rosettes from which cellulose is produced (Arioli et al., 1998). The RSW1 gene encodes a distant relative of the catalytic subunit of bacterial cellulose synthases with predicted eight membrane-spanning domains (Arioli et al., 1998).

Screens for abnormal root cell expansion have also identified several mutations that affect root cell shape in other ways. For the CORE (Conditional Root Expansion) class of mutants the cell expansion phenotype is conditional upon growth rate – under conditions that lower the growth rate (lower sucrose or reduced temperature) the root cells resemble wild type (Hauser et al., 1995). In one of the CORE mutants, *cobra* (*cob*), the primary defect appears to be in the regulation of the orientation of expansion. In contrast, the *lion's tail* mutation causes reduced elongation with a slight increase in radial expansion so that cell volume is significantly less than wild type. The other four CORE mutations, *quill*, *cudgel*, *pom-pom1* and *pom-pom2* have cell volumes that are greater than wild type which would be consistent with a structural role, or a role in regulating the extent of expansion (Hauser et al., 1995).

Two of the original CORE mutants were picked up in other genetic screens. The *procuste* mutation was identified in a screen for abnormal hypocotyl elongation and is allelic to *quill* (Desnos et al., 1996). *PROCUSTE/QUILL* encodes another catalytic subunit of the cellulose synthesis enzymatic complex (Fagard et al., 2000). The *korrigan* mutation was isolated in a screen of insertional mutants for short and radially expanded hypocotyl cells and is allelic to *lion's tail*. *KORRIGAN* encodes a member of the endo-1,4-b-D-glucanase (EGase) family (Nicol et al., 1998; Sato et al., 2001). The deduced amino acid sequence of KOR indicates that it is an integral membrane protein with its catalytic domain external to the membrane.

Analysis of cellulose synthesis in *cobra* revealed a deficit of cellulose in the root suggesting that COB's regulation of oriented cell expansion is associated with the deposition of cellulose. Sequence motifs indicate that the C-terminus of COBRA is processed and replaced with a glycosylphosphatidylinositol (GPI) anchor (Schindelman et al., 2001). In animal cells, this lipid linkage is known to confer polar localization to proteins. Antibodies localize COB protein predominately on the longitudinal sides of root cells in the zone of rapid elongation consistent with a role in regulating the orientation of cell expansion (Fig. 14). mRNA expression levels of COBRA increase dramatically in cells entering the zone of rapid longitudinal expansion (Schindelman et al., 2001) (Fig. 15).

References:

Arioli, T., Peng, L., Betzner, A. S., Burn, J., Wittke, W., Herth, W., Camilleri, C., Hofte, H., Plazinski, J., Birch, R.*, et al.* (1998). Molecular analysis of cellulose biosynthesis in Arabidopsis, Science **279**, 717-20.

Cnops, G., Wang, X., Linstead, P., Van Montagu, M., Van Lijsebettens, M., and Dolan, L. (2000). Tornado1 and tornado2 are required for the specification of radial and circumferential pattern in the Arabidopsis root, Development **127**, 3385-94.

Desnos, T., Orbovic, V., Bellini, C., Kronenberger, J., Caboche, M., Traas, J., and Hofte, H. (1996). Procuste1 mutants identify two distinct genetic pathways controlling hypocotyl cell elongation, respectively in dark- and light-grown Arabidopsis seedlings, Development **122**, 683-93.

Di Laurenzio, L., Wysocka-Diller, J., Malamy, J. E., Pysh, L., Helariutta, Y., Freshour, G., Hahn, M. G., Feldmann, K. A., and Benfey, P. N. (1996). The SCARECROW gene regulates an asymmetric cell division that is essential for generating the radial organization of the Arabidopsis root, Cell **86**, 423-33.

Dolan, L., Janmaat, K., Willemsen, V., Linstead, P., Poethig, S., Roberts, K., and Scheres, B. (1993). Cellular organisation of the Arabidopsis thaliana root, Development **119**, 71-84.

Fagard, M., Desnos, T., Desprez, T., Goubet, F., Refregier, G., Mouille, G., McCann, M., Rayon, C., Vernhettes, S., and Hofte, H. (2000). PROCUSTE1 encodes a cellulose synthase required for normal cell elongation specifically in roots and dark-grown hypocotyls of Arabidopsis, Plant Cell **12**, 2409-2424.

Hauser, M. T., Morikami, A., and Benfey, P. N. (1995). Conditional root expansion mutants of Arabidopsis, Development **121**, 1237-52.

Helariutta, Y., Fukaki, H., Wysocka-Diller, J., Nakajima, K., Jung, J., Sena, G., Hauser, M.-T., and Benfey, P. N. (2000). The SHORT-ROOT Gene Controls Radial Patterning of the Arabidopsis Root through Radial Signaling, Cell **101**, 555-567.

Inoue, T., Higuchi, M., Hashimoto, Y., Seki, M., Kobayashi, M., Kato, T., Tabata, S., Shinozaki, K., and Kakimoto, T. (2001). Identification of CRE1 as a cytokinin receptor from Arabidopsis, Nature **409**, 1060-3.

Mahonen, A. P., Bonke, M., Kauppinen, L., Riikonen, M., Benfey, P. N., and Helariutta, Y. (2000). A novel two-component hybrid molecule regulates vascular morphogenesis of the Arabidopsis root, Genes Dev **14**, 2938-43.

Nakajima, K., Sena, G., Nawy, T., and Benfey, P. N. (2001). Intercellular movement of the putative transcription factor SHR in root patterning, Nature **413**, 307-11.

Nicol, F., His, I., Jauneau, A., Vernhettes, S., Canut, H., and Hofte, H. (1998). A plasma membrane-bound putative endo-1,4-beta-D-glucanase is required for normal wall assembly and cell elongation in Arabidopsis, Embo J **17**, 5563-76.

Sato, S., Kato, T., Kakegawa, K., Ishii, T., Liu, Y. G., Awano, T., Takabe, K., Nishiyama, Y., Kuga, S., Nakamura, Y.*, et al.* (2001). Role of the putative membrane-bound endo-1,4-beta-glucanase KORRIGAN in cell elongation and cellulose synthesis in Arabidopsis thaliana, Plant Cell Physiol ***42***, 251-63.

Scheres, B., Di Laurenzio, L., Willemsen, V., Hauser, M.-T., Janmaat, K., Weisbeek, P., and Benfey, P. N. (1995). Mutations affecting the radial organisation of the Arabidopsis root display specific defects throughout the embryonic axis, Development **121**, 53-62.

Schindelman, G., Morikami, A., Jung, J., Baskin, T. I., Carpita, N. C., Derbyshire, P., McCann, M. C., and Benfey, P. N. (2001). COBRA encodes a putative GPI-anchored protein, which is polarly localized and necessary for oriented cell expansion in Arabidopsis, Genes Dev **15**, 1115-27.

Wysocka-Diller, J. W., Helariutta, Y., Fukaki, H., Malamy, J. E., and Benfey, P. N. (2000). Molecular analysis of SCARECROW function reveals a radial patterning mechanism common to root and shoot, Development **127**, 595-603.

Alvarez-Buylla ER, Liljegren SJ, Pelaz S, Gold SE, Burgeff C, Ditta GS, Vergara-Silva F, Yanofsky MF (2000). MADS-box gene evolution beyond flowers: expression in pollen, endosperm, guard cells, roots and trichomes. Plant J. **24**(4):457-66.

Baumberger, N., Ringli, C., Keller, B. (2001). The chimeric leucine-rich repeat/extensin cell wall protein LRX1 is required for root hair morphogenesis in Arabidopsis thaliana. Genes Dev. **15**, 1128-39.

Beemster, G.T.S., Baskin, T.I. (1998) Analysis of cell division and elongation underlying the developmental acceleration of root growth in *Arabidopsis thaliana* Plant Physiol. **116**, 1515-1526.

Berger F, Hung CY, Dolan L, Schiefelbein J. (1998) Control of cell division in the root epidermis of Arabidopsis thaliana. Dev Biol. **194**, 235-45.

Berger, F., Haseloff, J., Schiefelbein, J., Dolan, L.. (1998) Positional information in root epidermis is defined during embryogenesis and acts in domains with strict boundaries. Curr Biol. **8**, 421-30.

Berleth, T., and Jurgens, G. (1993). The role of the *monopteros* gene in organising the basal body region of the *Arabidopsis* embryo. Development **118**, 575-587.

Boerjan W, Cervera MT, Delarue M, Beeckman T, Dewitte W, Bellini C, Caboche M, Van Onckelen H, Van Montagu M, Inze D. (1995). Superroot, a recessive mutation in Arabidopsis, confers auxin overproduction. Plant Cell **7**(9):1405-19.

Casimiro I, Marchant A, Bhalerao RP, Beeckman T, Dhooge S, Swarup R, Graham N, Inze D, Sandberg G, Casero PJ, Bennett M. (2001). Auxin transport promotes arabidopsis lateral root initiation. Plant Cell. **13**(4):843-52.

Celenza JL Jr, Grisafi PL, Fink GR (1995). A pathway for lateral root formation in Arabidopsis thaliana. Genes Dev 9(17):2131-42.

Clowes, F.A.L. (1954) The promeristem and minimal construction centre in grass roots. New Phytol. **52**, 48-57.

Dolan, L., Duckett, C.M, Grierson, C., Linstead, P., Schneider, K., Lawson, E., Dean, C., Poethig, R.S., Roberts, K. (1994) Clonal relationships and cell patterning in the root epidermis of *Arabidopsis. Development* **120**, 2465-2475.

Dolan,L, Janmaat,K., Willemsen,V., Linstead,P., Poethig,S., Roberts,R., and Scheres,B. (1993). Cellular organisation of the *Arabidopsis thaliana* root. Development **119**, 71-84.

Favery, B., Ryan, E., Foreman, J., Linstead, P., Boudonck, K., Steer, M., Shaw, P., Dolan, L. (2001) *KOJAK* encodes a cellulose synthase-like protein required for root hair cell morphogenesis in Arabidopsis. Genes Dev. **15**, 79-89.

Friml J, Benkova E, Blilou I, Wisniewska J, Hamann T, Ljung K, Woody S, Sandberg G, Scheres B, Jürgens G, Palme K. (2002). AtPIN4 mediates sink-driven auxin gradients and root patterning in *Arabidopsis*. Cell, in press.

Fujie, M., Kuroiwa, H., Suzuki, T., Kawano, S., Kuroiwa, T. (1993) Organelle DNA synthesis in the quiescent centre of *Arabidopsis thaliana*. (Col.) Jour. Exp Botany **44**, 69-693.

Fukaki H, Tameda S, Masuda H, Tasaka M. (2002) Lateral root formation is blocked by a gain-of-function mutation in the SOLITARY-ROOT/IAA14 gene of Arabidopsis. Plant J. **29**, 153-68.

Galway, M.E., Masucci, J.D., Lloyd, A.M., Walbot, V., Davis, R.W., Schiefelbein J.W. (1994) The TTG gene is required to specify epidermal cell fate and cell patterning in the Arabidopsis root. Dev Biol. **166**, 740-54.

Hamann T, Mayer U, Jurgens G. (1999). The auxin-insensitive bodenlos mutation affects primary root formation and apical-basal patterning in the Arabidopsis embryo.Development 126(7):1387-95.

Hardtke, C.S., and Berleth, T. (1998). The *Arabidopsis* gene *MONOPTEROS* encodes a transcription factor mediating embryo axis formation and vascular development. EMBO J. **17**, 1405-1411.

Hobbie L, McGovern M, Hurwitz LR, Pierro A, Liu NY, Bandyopadhyay A, Estelle M. (2000). The axr6 mutants of Arabidopsis thaliana define a gene involved in auxin response and early development. Development **127**(1):23-32.

Kidner, C., Sundaresan, V., Roberts, K., Dolan, L. (2000) Clonal analysis of the Arabidopsis root confirms that position not lineage determines cell fate. Planta **211,** 191-199

Lee MM, Schiefelbein J (1999) WEREWOLF, a MYB-related protein in Arabidopsis, is a position dependent regulator of epidermal cell patterning. *Cell* **99**, 473-483.

Leyser, O., Lincoln, C.A., Timpte, C., Lammer, D., Turner, J., and Estelle M. (1993). *Arabidopsis* auxin-resistance gene *AXR1* encodes a protein related to ubiquitin-activating enzyme E1. Nature **364**, 161-164.

Malamy JE, Benfey PN. (1997) Organization and cell differentiation in lateral roots of Arabidopsis thaliana. Development **124**(1):33-44.

Masucci, J.D., Rerie, W.G., Foreman, D.R., Zhang, M., Galway, M.E., Marks. M.D., Schiefelbein, J.W. (1996) The homeobox gene GLABRA2 is required for position-dependent cell differentiation in the root epidermis of *Arabidopsis thaliana*. Development. **122**, 1253-60.

Mayer KF, Schoof H, Haecker A, Lenhard M, Jurgens G, Laux T. (1998). Role of WUSCHEL in regulating stem cell fate in the Arabidopsis shoot meristem. Cell **95**(6):805-15.

Ouellet F, Overvoorde PJ, Theologis A. (2001). IAA17/AXR3. Biochemical insight into an auxin mutant phenotype. Plant Cell **13**(4):829-42.

Parker, J.S., Cavell, A.C., Dolan, L., Roberts, K., Grierson, C.S. (2000) Genetic interactions during root hair morphogenesis in Arabidopsis. Plant Cell. **12**, 1961-74.

Rigas, S., Debrosses, G., Haralampidis, K., Vicente-Agullo, F., Feldmann,.K., Grabov, A., Dolan, L., Hatzopoulos, P. (2001) *TRH1* encodes a potassium transporter required for tip growth in arabidopsis root hairs. Plant Cell. **13**,139-51.

Rouse D, Mackay P, Stirnberg P, Estelle M, Leyser O. (1998) Changes in auxin response from mutations in an AUX/IAA gene. Science **279**(5355):1371-3.

Ruegger, M., Dewey, E., Gray, W.M., Hobbie, L., Turner, J., and Estelle, M. (1998). The TIR1 protein of *Arabidopsis* functions in auxin response and is related to human SKP2 and yeast grr1p. Genes Dev. **12**, 198-207.

Sabatini, S., Beis, D., Wolkenfelt, H., Murfett, J., Guilfoyle, T., Malamy, J., Benfey, P., Leyser, O., Bechtold, N., Weisbeek P. and Scheres, B. (1999). An auxin-dependent distal organizer of pattern and polartity in the *Arabidopsis* root. Cell **99**, 463–472.

Scheres, B., Wolkenfelt. H., Willemsenm V., Terlouw, M.P., Lawson, E., Dean, C., Weisbeek, P. (1994) Embryonic origin of the Arabidopsis primary root and root meristem initials. Development **120**, 2475-2487.

Scheres,B., DiLaurenzio,L, Willemsen,V., Hauser,M.-T., Janmaat, K., Weisbeek,P. and Benfey,P (1995) Mutations affecting the radial organisation of the *Arabidopsis* root display specific defects throughout the embryonic axis. Development **121**, 53-62.

Schiefelbein, J.W., Somerville, C. (1990) Genetic control of root hair development in *Arabidopsis thaliana*. Plant. Cell **2**, 235-243.

Schneider K, Mathur J, Boudonck K, Wells B, Dolan L, Roberts K (1998) *The ROOT HAIRLESS 1* gene encodes a nuclear protein required for root hair initiation in Arabidopsis. Genes Dev. **12**, 2013-21.

Schneider, K., Wells, B., Dolan, L., & Roberts, K. (1997) Structural and genetic analysis of epidermal cell differentiation in *Arabidopsis* primary roots. Development **124**, 1789-1798.

Steinmann T, Geldner N, Grebe M, Mangold S, Jackson CL, Paris S, Galweiler L, Palme K, Jurgens G. 1999 Coordinated polar localization of auxin efflux carrier PIN1 by GNOM ARF GEF. Science **286**(5438):316-8.

Timpte C, Wilson AK, Estelle M. (1994). The axr2-1 mutation of Arabidopsis thaliana is a gain-of-function mutation that disrupts an early step in auxin response. Genetics **138**(4):1239-49.

Van den Berg, C., Willemsen, V., Hendriks, C., Weisbeek, P. and Scheres, B. (1997). Short-range control of cell differentiation in the *Arabidopsis* root meristem. Nature **390**, 287-289.

Van den Berg, C., Willemsen,V., Hage,W., Weisbeek,P. and Scheres,B. (1995) Cell fate in the *Arabidopsis* root determined by directional signalling. Nature **378**, 62-65.

Wada T, Tachibana T, Shimura Y and Okada K. (1997). Epidermal cell differentiation in Arabidopsis determined by a Myb homolog, CPC. Science **277**, 1113-1116.

Wang, H., Lockwood, S.K., Hoeltzel, M.F., Schiefelbein, J.W. (1997) The ROOT HAIR DEFECTIVE3 gene encodes an evolutionarily conserved protein with GTP-binding motifs and is required for regulated cell enlargement in Arabidopsis. Genes Dev. **11**, 799-811.

Willemsen, V., Wolkenfelt, H., de Vrieze, G, Weisbeek, P. and Scheres, B. (1998). The *HOBBIT* gene is required for formation of the primary root meristem in the *Arabidopsis* embryo. Development **125**, 521-531.

Wymer CL, Bibikova TN, Gilroy S: Cytoplasmic free calcium distributions during the development of root hairs of Arabidopsis thaliana. 1997*, Plant J.* **12**:427-39.

Xie Q, Frugis G, Colgan D, Chua NH. (2000). Arabidopsis NAC1 transduces auxin signal downstream of TIR1 to promote lateral root development. Genes Dev. **14**(23):3024-36.