Cell signaling in root development

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Cell signaling has recently been shown to be of major importance in cell specification during *Arabidopsis* root development. In the ground tissue, cues of unknown molecular nature convey positional information and two genes provide an interesting link between asymmetric cell division and the determination of cell fate. In the root epidermis, cell specification involves ethylene signaling and transcription factors of which at least two are also required for cell fate decisions in the shoot epidermis.

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Abbreviations

ACC	1-aminocyclopropane-1-carboxylate
AVG	aminoethoxyvinyl glycine
CTR1	CONSTITUTIVE TRIPLE RESPONSE 1
eto	*ethylene overproducing*
GL2	*GLABRA2*
SCR	*SCARECROW*
SHR	*SHORTROOT*
TTG	*TRANSPARENT TESTA GLABRA*

Introduction

Anyone looking around in a flower shop may notice ornamental plants chimeric for pigmentation traits, their colour patterns a vivid display of cell lineage variability. Indeed, numerous studies of plant development indicate that cell lineage is, in most cases, variable and yet regular patterns of specialized cells arise [1]. As plant cells cannot undergo active movement, it is only common sense to suspect a key role for cell signaling in pattern formation and to presume that signaling takes place within the continuously developing regions of the plant — the meristems.

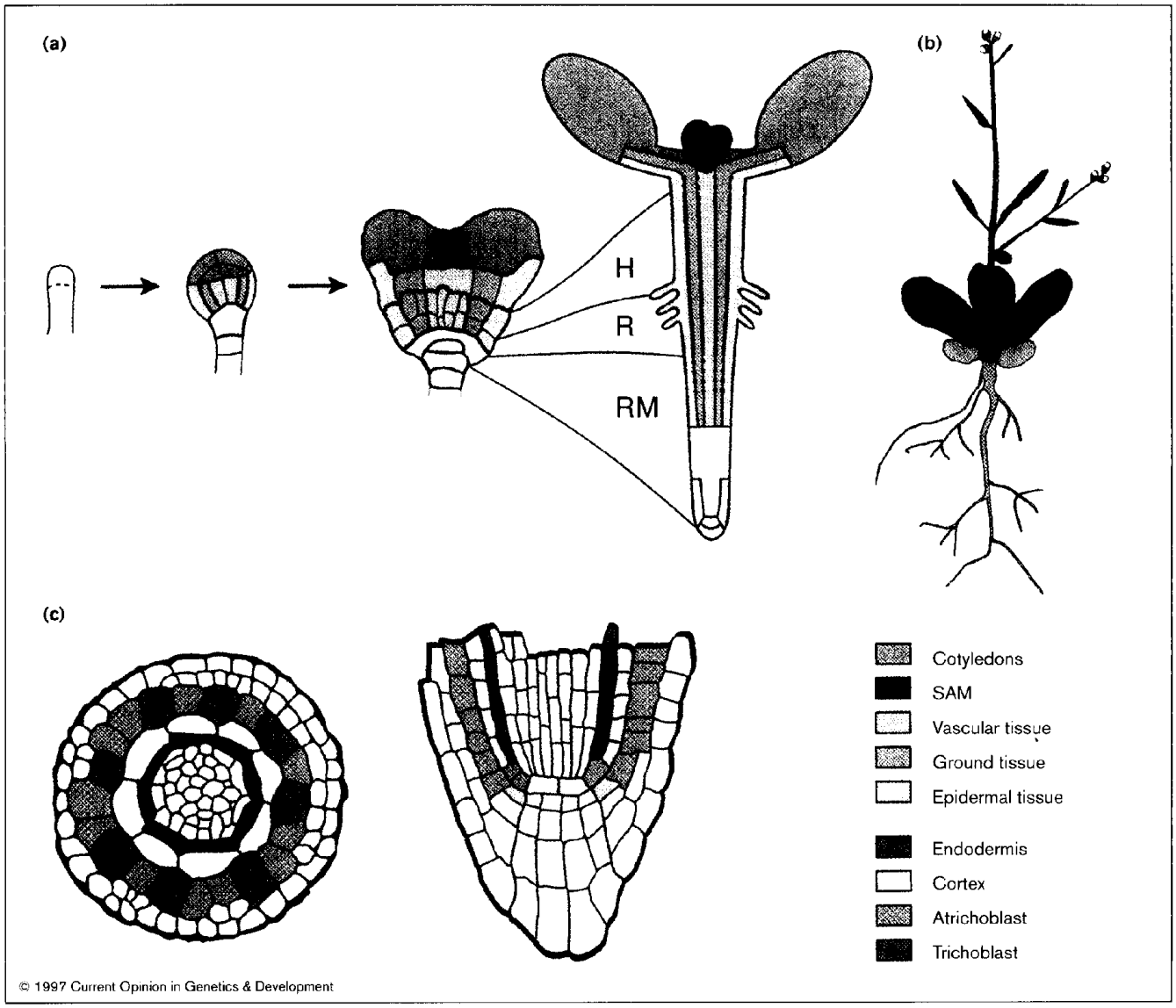
Within floral meristems, transcription factors are known to be involved in regional specification [2]; much less is known, however, about gene products that are required for signaling processes during pattern formation. Recently, a putative transmembrane kinase involved in cell differentiation and a candidate peptide growth factor have been discovered in plants, implying that intercellular signals similar to those employed in animals operate during plant development [3,4]. Nevertheless, surprisingly few studies on signaling pathways involved in plant cell specification exist that combine both genetic and experimental approaches. It has become clear recently that the *Arabidopsis thaliana* root allows such combined studies because of its regular cellular architecture, small size, and transparency. In this review, I focus on research carried out in the past two years on two examples of cell specification in the *Arabidopsis* root and sketch interpretations that may stimulate thought on the role of intercellular communication in plant cell specification.

Cortex or endodermis: positional cues and asymmetric divisions

The radial pattern of cell types in the *Arabidopsis* root reflects the organization of the three main tissues in the embryo axis (Figure 1) [5]. In the axial direction, cells are arranged in regular files that are extended at their basal ends by stereotyped cell divisions in the root meristem (Figure 1c). The distal-most root cap is produced by a separate set of meristem cells. Fate-mapping studies have revealed that the cell lineage of the root is largely invariant [6] — suggesting cell-autonomous development — but laser ablation studies demonstrate that positional signals are decisive for the determination of cell fate (see below). The specification of two ground-tissue-derived cell types — the cortex (cortical parenchyma) and the endodermis — serves well to illustrate this.

During embryogenesis, the ground tissue divides into two layers in the root, the innermost layer being the prospective endodermis (Figure 1c). Within the root meristem, the basal-most ground tissue cells usually remain undivided and they act as initial (stem) cells for cortex and endodermis. These initial cells continuously add new cells to the ground tissue. The daughters of the initial cells divide asymmetrically to form cortical and endodermal cells (after a few proliferative divisions) (Figures 1c and 2a). Laser ablation experiments have demonstrated that cells derived from a neighbouring tissue can switch fate in response to positional cues when they become located in the ground tissue layer ([7]; Figure 3a–c). Blocking contacts between an initial cell and more mature daughter cells by laser ablation specifically inhibits the asymmetric division, giving rise to cortex and endodermis [7]. It was concluded that the initial cell requires signals to perform the asymmetric division and that these signals seem to originate from overlying more mature cells of the same tissue (Figure 3c). A recent study on plasmodesmal density and cell connectivity in the *Arabidopsis* root indicates that cells are most efficiently connected within their own tissue layer (T Zhu, WJ Lucas, T Rost, personal communication). In the epidermal layer, this connectivity persists until the cells are fully differentiated [8]. One could speculate that such a plasmodesmal network provides a structural framework for transport of tissue-specific specification signals.

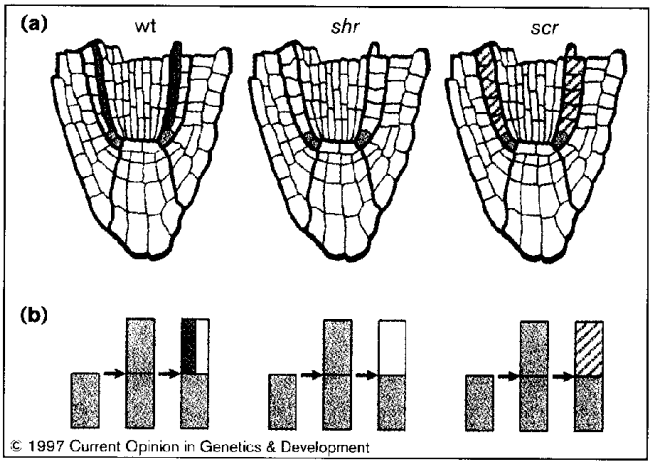
Figure 1



Ontogeny of meristems in *Arabidopsis*. **(a)** Embryogenesis results in a seedling containing the primary shoot apical meristem and derived leaves (SAM), the root meristem (RM) as well as three organ systems in the main axis: embryonic leaves (cotyledons), embryonic root (R) and hypocotyl (H). The vascular, ground and epidermal tissues are shared by all organs, which are depicted only in the middle sections of the drawings. **(b)** The adult plant develops from the primary meristems and from newly formed secondary meristems. **(c)** Cross section and median longitudinal section through root meristem highlighting the epidermis-derived trichoblast and atrichoblast cells, and ground-tissue-derived cortex and endodermis.

Which genes are involved in the specification of cortex and endodermis? Plants homozygous for recessive mutations in the *SHORTROOT* (*SHR*) and *SCARECROW* (*SCR*) genes lack at least one ground tissue layer throughout the embryo axis [9,10]. In the root of *shr* mutants, the single remaining layer is lacking endodermal markers. In roots of *scr* mutants, a layer with mixed cortical/endodermal attributes remains ([11••]; Figure 2). The mixed cell type in *scr* mutants is unusual and interesting as mutations that interfere with asymmetric cell division in yeast and animals generally result in cells of one type [12]. The *shr* and *scr* phenotypes imply that the product of the *SHR* gene is required to determine endodermal cell fate as well as for the asymmetric division and that the *SCR* gene is required specifically for the asymmetric division [11••]. Perhaps the *SCR* gene is required exclusively for polarization of the cell and subsequent execution of an asymmetric division in a direction perpendicular to the proliferative divisions in the meristem (Figure 2b). In the brown alga *Fucus*, as well as in *Caenorhabditis elegans*, *Drosophila*, and mammals, polarized distribution of the cell fate determinants and alignment of cell division plane have been shown to be linked to the same underlying mechanism of polarity establishment ([13,14] and reference therein). The observation that *SHR* is also required for asymmetric division may indicate that this

Figure 2



(a) *shortroot* (*shr*) and *scarecrow* (*scr*) mutants have altered cell fate and lack asymmetric cell division in the ground tissue layer. **(b)** Schematized division patterns and fate of cortex/endodermis initial cells in wild-type and mutant meristems.

gene is an upstream regulator of *SCR*. The *SCR* gene has been cloned recently; it encodes several structural motifs, suggesting that the product is a transcription factor, and is expressed in the initial cells and throughout the endodermis in the meristem [11••].

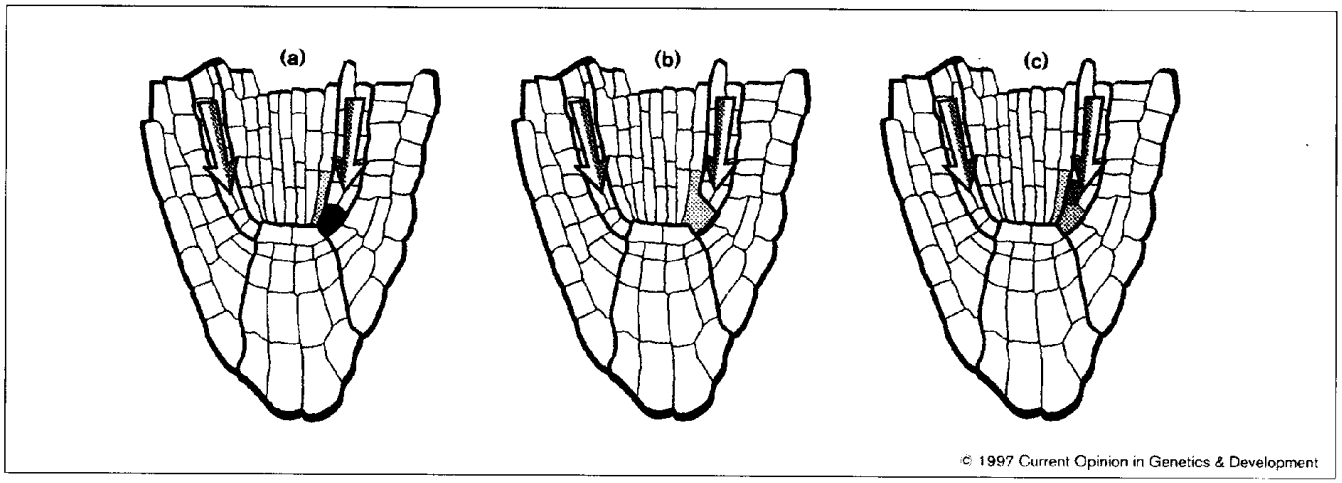
Clarification is needed of how SCR function as a transcriptional regulator is related to cell division and the functional significance of persistent *SCR* expression in the endodermis. Targeted expression of the *SCR* gene in a *scr* mutant background should reveal whether endodermal expression of *SCR* is of relevance for the asymmetric division of the initial cell. If so, the gene may regulate synthesis of the signal inferred from the laser ablation experiments mentioned above that triggers asymmetric division non-cell-autonomously. Taking this line of thought one step further by considering the observation that transcription factors can move through plasmodesmata ([15]; Jackson and Hake, this issue, pp 495–500) a more tantalizing possibility is that the *SCR* gene encodes the signal. Regardless of the eventual outcome, analysis of the relationship between the demonstrated signals and the *SCR* and *SHR* genes seems a promising route towards a better understanding of signaling processes in plant cell specification.

Hairs or no hairs

Although signals involved in specifying the cortex/endodermis have not yet been characterized, cell specification within the epidermal layer is known to involve the phytohormone ethylene. This molecule acts as a signal in a variety of processes and corresponding receptors and signal transducers have been identified [16]. The epidermal cell layer of the *Arabidopsis* root comprises hair-bearing (trichoblast) and hairless (atrichoblast) cells in files along the main axis. Hair cell files form at predictable positions over the anticlinal walls of underlying cortex cells [5] (Figures 1c and 4). The consistency of this position-dependent distribution has enabled the analysis of trichoblast patterning.

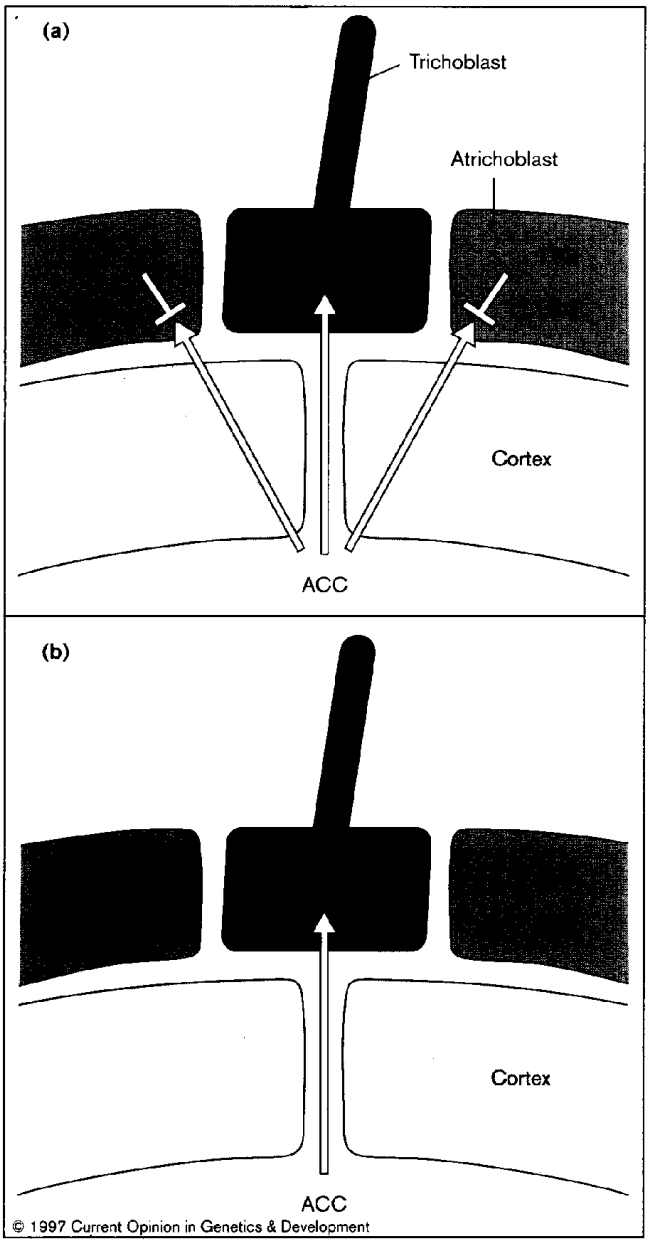
A number of studies demonstrate collectively that ethylene positively regulates trichoblast development. First, ectopic root hairs are formed upon addition of the ethylene precursor 1-aminocyclopropane-1-carboxylate (ACC) in *ethylene overproducing (eto)* mutants and in plants homozygous for mutant alleles of the *CONSTITUTIVE TRIPLE RESPONSE1* (*CTR1*) gene, which encodes a *RAF* homologue negatively regulating ethylene signal

Figure 3



Positional cues in the *Arabidopsis* root. **(a–c)** Schematic representation of a laser ablation experiment. (a) Ground tissue initial ablation (black). (b) Invasion of cells from neighbouring tissue (red). (c) Daughters of invaded cells switch fate and act as ground tissue initials. The arrows indicate the proposed direction of positional cues.

Figure 4



Models for the roles of negative (GL2) and positive (ethylene) inputs on trichoblast patterning. **(a)** Ethylene precursor (ACC) moves freely (arrows): ethylene activity is patterned by TTG and GL2 activity. **(b)** The ethylene precursor moves more readily through anticlinal clefts: ethylene pathway conveys independent patterning information.

transduction ([17,18]; XF Cao, L Dolan, personal communication). Second, a decrease in the number of hair cells is observed upon blocking either the synthesis or perception of ethylene with aminoethoxyvinyl glycine (AVG) and Ag^+, respectively ([19]; XF Cao, L Dolan, personal communication).

Mutants lacking leaf hair cells have recently provided a convenient shortcut to additional genes involved in trichoblast patterning. Plants homozygous for recessive mutations in the *TRANSPARENT TESTA GLABRA (TTG)* and *GLABRA2 (GL2)* genes were found to possess ectopic root hairs on all epidermal cell files [20,21•]. The *TTG* and *GL2* genes are thus required for negative regulation of trichoblast development in the non-hair-cell files. Surprisingly, the negative role of these genes in root hair cell specification contrasts with their positive role in leaf hair cell development.

The maize *R* gene—encoding a *myc* transcription factor—can complement the *ttg* mutation, indicating that the *TTG* gene either encodes or activates the *Arabidopsis* homologue of this transcription factor [22]. The *GL2* gene encodes a homeodomain-containing a putative transcription factor [23]. *GL2* mRNA levels are positively regulated by TTG [24]. Recently, the *CAPRICE* gene, encoding a protein with putative Myb DNA-binding motif but without an acidic activator domain, has been implied as an upstream *GL2* repressor (T Wada, Y Shimura, K Okada, personal communication). At least three interacting transcription factors are thus involved in atrichoblast specification.

To understand trichoblast patterning, it is important to locate the *TTG/GL2* and ethylene activities both spatially and temporally. On the basis of *in situ* hybridization and reporter gene fusions, the *GL2* gene is preferentially expressed in atrichoblast cell files well into the meristem region, consistent with a role in negative regulation of hair development in atrichoblasts [21•,25•]. Treatment of *ttg* and *gl2* mutants with the ethylene blocker AVG leads to the reduction of root hairs in all cell files, suggesting that the ethylene pathway is negatively regulated by TTG and GL2 in atrichoblast cells ([26••]; Figure 4a). Timing experiments indicate that ethylene overproduction triggers ectopic root hair formation after the cessation of cell division, whereas *ttg* and *gl2* mutants display a phenotype in the meristematic region ([26••]; XF Cao, L Dolan, personal communication). Taken together, the data suggest that ethylene appears to act at a late stage to promote root hair formation in cells where TTG/GL2 activity is either low or absent.

The possibility that ethylene also conveys positional information independently of TTG/GL2 activity deserves attention, as it is found that the remaining hairs in AVG-treated *ttg/gl2* mutants form preferentially in the correct position [17]. This observation indicates that either endogenous ethylene production or the sensitivity of ethylene reception are patterned in the absence of *ttg/gl2* activity. An ethylene distribution pattern is conceivable as diffusion of the ethylene precursor ACC towards the epidermis may be facilitated through the anticlinal clefts over which trichoblasts normally reside ([27]; L Dolan, personal communication) (Figure 4b). If there are two prepatterns, an important issue is how they are put into register such that cells overlying cortical clefts respond to ethylene more readily and the remaining cells express

GL2. One possibility, on the basis of differential epidermal sensitivity to ethylene, is that CAPRICE or similar gene products may be induced in trichoblast precursor cells by cues from the cortical clefts and subsequently set up both patterns by repressing *GL2* and simultaneously enhancing ethylene sensitivity.

Conclusions

Recent studies on cell specification in *Arabidopsis* roots are beginning to shed light on the role of intercellular communication in the determination of plant cell fate. In the ground tissue, hitherto uncharacterized signals appear to convey positional information and two genes have been identified that highlight an interesting and possibly plant-specific relationship between cell fate and asymmetric cell division. Trichoblast specification involves the relatively well characterized ethylene signaling pathway and a set of transcription factors also utilized in leaf hair formation. We may soon learn whether ethylene is a permissive signal or whether it also conveys positional information. We do not yet know enough of the two demonstrated signaling events to describe them in mechanistic detail but the experimental advantages of studying roots and the ongoing improvements of the *Arabidopsis* genetic model system may change this situation in the near future.

Acknowledgements

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