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# The Development of Cell Pattern in the Root Epidermis

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*Phil. Trans. R. Soc. Lond. B* 1995 **350**, 95-99
doi: 10.1098/rstb.1995.0143

# The development of cell pattern in the root epidermis

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## SUMMARY

The root epidermis of most angiosperms is composed of a patterned array of hair and non-hair cells. Hair cells may develop randomly in any location in the epidermis (type 1), from specialized cells that form as result of an asymmetric cell division in a mother cell (type 2) or cells may be arranged in files of one cell type or the other (type 3). The development of the epidermis in *Arabidopsis* has been examined in detail and corresponds to type 3 epidermal development. A combination of physiological and genetic observations indicates that ethylene is a positive regulator of root hair differentiation. Differential exposure of epidermal cells to ethylene as a result of the cellular geometry of the root may account for the wild-type epidermal pattern.

## 1. INTRODUCTION

The generation of pattern in developing systems involves an interplay of cell autonomous and cell non-autonomous factors. In other words, there are diffusible signals (cell non-autonomous factors) that emanate from a site and elicit effects at some distance from this site by interacting with cells with appropriate receptors (cell autonomous factors). Simplistically, one can say that it is the relative spatial relations between these two factors that are responsible for the patterns that 'development' produces. The patterned elements that result may be on a variety of scales. A large scale pattern might include the branching pattern of a tree that constitutes its architectural model (Halle *et al.* 1978). At a smaller scale one can consider the relatively invariant organisation of leaves around a shoot of any species at any given stage in its development (Steeves & Sussex 1989). At an even smaller scale one can consider the organisation of cells that comprise a tissue, for example the patterned array of trichomes on a leaf (Hülskamp *et al.* 1994). In each case, however, the pattern is initially established at the cellular level and over relatively small distances ($< 1$ mm). Although these three examples of developmental pattern may seem disparate, they have at least one factor in common: in each system we are considering how cells either in groups or as individuals organise themselves into coherent structures in the mature plant. It is the aim of this paper to describe the current state of our understanding of the factors that are involved in organising the cells of the *Arabidopsis* root epidermis into a patterned array.

## 2. THE ROOT EPIDERMIS IS GENERALLY COMPOSED OF A PATTERNED ARRAY OF HAIR AND NON-HAIR CELLS

The root epidermis of most higher plants is composed of two cell types: hair cells that are derived from trichoblasts and non-hair cells that are derived from atrichoblasts. Hair cells are specialized epidermal cells that produce a tip-growing extension (a hair) from their outer face. These hair cells are interspersed between non-hair cells which generally have no obvious distinguishing features. The spatial organisation of these two cell types in the epidermis is species specific under controlled conditions, but numerous studies have shown that the pattern can be subtly changed by environmental factors (Abeles 1973). An extreme example of such a change is the dramatic alteration in root architecture and the suppression of root hair development found in species that form symbiotic associations with soil fungi, the so-called mycorrhizae (Peterson 1992).

The organisation of hair cells in the epidermis may be considered as falling into three broad groups.

1. Type 1: hair cells are randomly arranged in the epidermis. The development of hairs from non-specialized cells in a seemingly random pattern in the epidermis is a characteristic of most if not all dicot families except the Brassicaceae and Nymphaeaceae (Leavitt 1904). This pattern of development is to be observed in a number of monocot families. Within the Poaceae the subfamilies Bambusoideae, Arundinoideae, Chloridoideae and Panicoideae exhibit this pattern (Row & Reeder 1957; Clarke *et al.* 1979).

2. Type 2: hair cells are derived from specialized trichoblasts, the smaller product of an asymmetric cell division. Root-hair development in the root epidermis of the monocotyledonous, water weed *Hydrocharis morsusranae* is one of the best characterized examples of this type of root hair development (Cutter & Feldman 1970; Cutter & Hung 1972). Cells in the epidermis of this plant undergo asymmetric cell divisions producing two daughter cells. The smaller, more densely cytoplasmic cell undergoes no further cell divisions but goes on to form a hair. The larger daughter undergoes further rounds of cell division producing a clone of non-hair cells. Hairs are therefore spaced between a small number of non-hair cells along any single epidermal cell file and root hairs form in all files of the epidermis i.e. there is no circumferential differentiation of the cell files. A variation on this theme is to be found

*Phil. Trans. R. Soc. Lond.* B (1995) **350**, 95–99
*Printed in Great Britain*

in the Pooideae, a monophyletic subfamily of the Poaceae in which an asymmetric cell division produces a small cell that forms a hair and a larger cell that elongates to form a non-hair cell without any further cell division (Sinnott 1939; Clayton & Renvoize 1986; Kellogg & Campbell 1987). Although root-hair development involving asymmetric cell division is found predominantly among the monocots it is also observed in the Nymphaeaceae, a dicotyledonous family that has recently been shown to be phylogenetically related to the monocots (Chase *et al.* 1994; Clowes 1994).

3. Type 3: hair cells are formed from specialized cells along discrete files. Members of the Brassicaceae exhibit a unique patterning of epidermal cells (Cormack 1935; Bünning 1951; Dolan *et al.* 1994; Galway *et al.* 1994). Root hairs form in files in which every cell develops as a hair cell. These hair files are interspersed with files containing only non-hair cells. Although the trichoblasts in the hair files are not derivatives of asymmetric cell divisions they are nonetheless smaller and more densely cytoplasmic than the atrichoblasts in the non-hair files.

Although these may be considered to be the three main patterns of root-hair development in higher plants there are, of course, some variations on the theme. For example, closer examination of root hair development in the Poaceae led Row & Reeder (1957) to conclude that some species such as *Eleusine indica* of the subtribe Chlorideae, exhibit a developmental pattern intermediate between the random pattern (Type1) and the highly specialized asymmetric cell division pattern (Type 2).

## 3. DEVELOPMENT OF THE ROOT EPIDERMIS IN *ARABIDOPSIS*

The epidermal initials (cells from which the epidermis is derived) of the *Arabidopsis* root are arranged in a ring below the central cells of the quiescent centre. Clonal analysis has revealed that these cells undergo a periclinal division, the outer derivative of which goes on to form the lateral root cap whereas the inner derivative regenerates the initial by transverse division and forms a clone of epidermal cells (Dolan *et al.* 1993, 1994). The ultimate fate of these epidermal cells depend on their position relative to underlying cortical cells (Dolan *et al.* 1994; Galway *et al.* 1994). Those cells that lie over the junction between two cortical cells differentiate as hair cells whereas those which lie over the outer wall of a single cortical cell differentiate as non-hair cells. Because there are always eight cortical cells in this root it follows that there are only eight hair files in the epidermis that varies from 16–23 cells in circumference (see figure 1).

The differentiation between the cell types is apparent before hairs appear. The shorter and more densely cytoplasmic trichoblast morphology is apparent soon after the cells emerge from below the disintegrating lateral root cap. In addition, the general shaping and tapering of the epidermal cells, to fit comfortably into the epidermal cylinder, also differs between hair and non-hair cells (see figure 1). The ratio of the lengths of outer-periclinal wall to inner-periclinal wall is 0.77 for hair cells (i.e. outwardly tapered) but 2.09 for non-hair cells (i.e. inwardly tapered), a significant difference. The 'shape factor' may prove useful in the analysis of mutants described later, to discriminate between cell types in the absence of hairs. Although the precise cellular geometry of the root predicts the fate of the cell, the signals that are involved in fate specification probably act sometime after the first periclinal division of the epidermal initial but before the differentiation of the epidermal cells into long and short cells as they emerge from beneath the root cap.

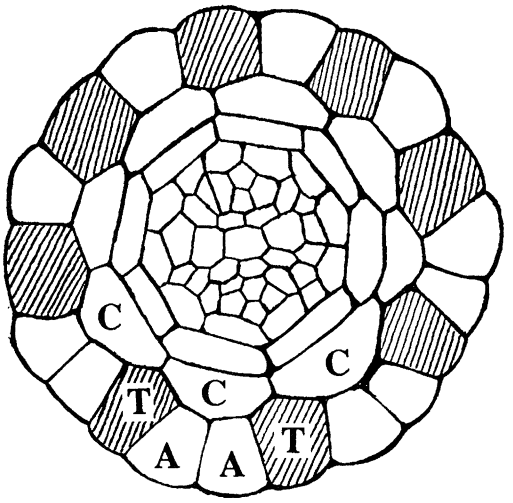



Figure 1. Schematic cross section of *Arabidopsis* primary root in the region just above the root meristem. The eight cells (or trichoblasts, T) that will eventually form hair- cells are shaded. The intervening cells are atrichoblasts (A). Trichoblasts are positioned over an anticlinal wall in between adjacent underlying cortical cells (C).

## 4. ETHYLENE IS A POSITIVE REGULATOR OF ROOT HAIR DEVELOPMENT IN DIVERSE GROUPS OF PLANTS

The plant growth factor ethylene is derived from methionine and is involved in a multitude of plant developmental processes (Yang & Hoffman 1984; Zarembinski & Theologis 1994; Ecker *et al.*, this volume). The rate-limiting step in the synthesis of ethylene is the formation of 1-amino-1-cyclopropane carboxylate(ACC) from S-adenosyl methionine by the action of ACC synthase (Adams & Yang 1979), ACC is in turn converted into ethylene by the action of ACC oxidase. A number of genes have been characterized that are involved in the perception of the ethylene, a process blocked by silver ions (Bleecker *et al.* 1988; Guzman & Ecker 1990; Van der Straeten *et al.* 1993; Roman *et al.* 1995). The *ETR1* gene encodes a protein similar to the bacterial two component kinases and the *CTR1* gene encodes another kinase with some similarity to the eukaryotic Raf1 family.

That ethylene can act as a positive regulator in root hair development was shown by Cormack in the 1930s (Cormack 1937). Trichoblasts are derived from the smaller product of an asymmetric cell division in the




PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

developing epidermis (Type 2 above) in soil grown *Elodea*. Roots of this species fail to develop root hairs when grown in water but can be induced to do so upon treatment with ethylene. Although root hairs develop in an apparently random fashion in the epidermis of pea, the growth of pea roots in the presence of ethylene appeared to increase the proportion of epidermal cells that developed root hairs (Abeles 1973). It is interesting that ethylene appears to be a positive regulator of root-hair development in these two species which exhibit two different root-hair developmental-pathways: one undergoing asymmetric cell division before the differentiation of the trichoblast (*Elodea*, type 2); and the other (pea) exhibiting no such apparent asymmetry (pea, type 1). It was therefore of interest to determine whether ethylene could induce the differentiation of root hairs in a species with the third pattern of root hair development where the epidermis is composed of files of hair cells and non-hair cells.

## 5. ETHYLENE IS A POSITIVE REGULATOR OF HAIR CELL DEVELOPMENT IN *ARABIDOPSIS*

To determine whether ethylene is a positive regulator of root-hair differentiation in *Arabidopsis* roots, seedlings were germinated in the presence of the ethylene precursor ACC (M. Tanimoto, K. Roberts & L. Dolan, in press). Although ACC treatment results in shorter roots by inhibiting cell elongation, it also results in hairs differentiating in the place normally occupied by non-hair cells (the ectopic location). The greater the concentration of ACC the greater the fraction of hair cells that develop in the 'ectopic' location.

To further investigate the role of ethylene in hair-cell development, seedlings were grown in the presence of amino vinyl glycine (AVG) and silver ions (Masucci *et al.* 1994; M. Tanimoto, K. Roberts & L. Dolan, in press). AVG inhibits the rate limiting step in ethylene biosynthesis, the formation of ACC catalysed by ACC synthase. Silver ions block the perception of ethylene (Beyer 1976). Both AVG and silver ions inhibit the development of root hairs in a dose-dependent manner. Although the development of hairs on treated roots was inhibited by AVG and $Ag^{+}$, the hairs in the collet region at the hypocotyl base were refractory to the treatments. It has been shown that hairs in this region of the root develop on all epidermal cells indicating that these cells respond to different cues or that the cues are less spatially restricted than in the remainder of the root.

These experiments indicate that exposure of cells in non-hair files to ethylene alone can alter their fate so that they differentiate as hair cells, suggesting that they are not exposed to an ethylene signal during normal development. Blocking the synthesis of ethylene in the root inhibits the differentiation of hair cells suggesting that an ethylene signal to these cells may be a normal part of their development.

Although these physiological experiments implicate a role for ethylene in the specification of patterned differentiation in the root epidermis the analysis of mutant genes lays the foundation for molecular analysis of the mechanism underpinning epidermal cell patterning. The mammoth task undertaken in identifying genes involved in ethylene signal-transduction (Roman *et al.* 1995; Ecker *et al.*, this volume) has provided a battery of mutants useful in our analysis.

The *ETR1* gene has been cloned and encodes a protein similar to the two component kinases from bacteria (Chang *et al.* 1993). It is a candidate ethylene receptor. *etr1* roots produce ectopic hairs (P. Linstead & L. Dolan, unpublished data). Another negative regulator of the ethylene response, *CTR1* has been cloned and encodes a kinase with sequence similarity to proteins from the Raf1 family (Kieber & Ecker 1993; Kieber *et al.* 1993). Because *CTR1* is a negative regulator of the ethylene response, loss of function alleles at this locus result in a constitutive ethylene response. Consistent with this interpretation is the observation that *ctr1* roots possess ectopic hairs (Dolan *et al.* 1994).

Although the phenotypes of *etr1* and *ctr1* roots clearly implicate ethylene in the development of cell pattern in the epidermis the examination of other mutant alleles at other loci in the ethylene signal transduction pathway will determine which of these genes are also involved and how.

## 6. OTHER GENES KNOWN TO BE INVOLVED IN THE DEVELOPMENT OF PATTERNING IN THE EPIDERMIS

Screening for mutations that have ectopic root hairs, decreased hair density or no hairs has identified a number of genes that are involved in the specification of cell patterning the root epidermis (figure 2).

### (*a*) *ttg*

*ttg* roots develop hairs in all cell files indicating that this gene is a negative regulator of hair development (Galway *et al.* 1994). It is interesting to note that these presumptive loss of function *ttg* alleles result in the loss of trichomes (shoot hairs) in the shoot suggesting that this gene is a positive regulator of hair development in the shoot but a negative regulator of hair development in the root. The organisation of cells at the root apex in *ttg* plants is abnormal but goes on to produce a root with normal cellular architecture. More detailed analysis of the lineages are necessary.

### (*b*) *35S-Lc*

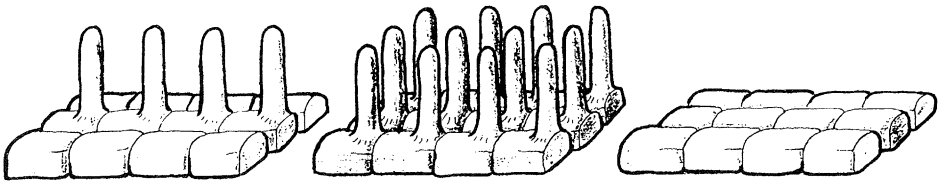
Plants transformed with the maize myb-like Lc gene under the control of the constitutive 35S promoter lack root hairs (Galway *et al.* 1994). Hemizygous plants develop more hairs than plants homozygous for the construct but fewer than wild type plants.

### (*c*) *dwf and* axr2

*dwarf* (*dwf*) plants are auxin resistant and produce a tiny root system that lack root hairs (Mizra *et al.* 1984).




PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES



(*a*) wild-type (*b*) *ttg, ctr1, etr1* (*c*) 35S-*Lc, dwf, axr2*

Figure 2. Schematic view of three epidermal cell files in the wild-type Arabidopsis primary root (a), in mutants with ectopic root hairs (b) and in mutants with no root hairs (c).

Auxin resistant 2 (*axr2*) plants are resistant to high levels of exogenous auxin, ethylene and abscisic acid in the root (Wilson *et al.* 1990). These roots lack hairs and are agravitropic.

## 7. MODEL

Our analysis of the development of cellular pattern in the *Arabidopsis* root epidermis has implicated ethylene as playing an important role. To briefly recap: treatment of roots with the ethylene precursor, ACC, results in the development of ectopic hairs. Cells in this ectopic location are therefore competent to respond to ethylene but during normal development may not receive such a signal. Treatment of roots with either AVG or $Ag^+$, which respectively inhibit ethylene synthesis or perception, blocks the formation of hairs on any cell. This indicates that during normal development trichoblasts receive an ethylene signal and disruption of that signal (by treatment with AVG or $Ag^+$) results in cells assuming the non-hair fate. The ectopic hair phenotypes of *etr1* and *ctr1* roots are consistent with these observations. Based on these data and assumptions we propose the following model. Epidermal cells are differentially exposed to ethylene with cells overlying the cleft between two underlying cortical cells exposed to the signal and cells overlying single cortical cells isolated from the signal. During the normal development of a trichoblast cell it is exposed to ethylene which inactivates ETR1 which then fails to activate CTR1, a repressor of the ethylene response, resulting in an activation of the ethylene signalling cascade. As cells in the position of non-hair cells are not exposed to ethylene (or to subthreshold levels) ETR1 activates CTR1 which represses the signalling cascade resulting in the development of a non-hair cell. The role of *TTG* in such a cascade requires its epistatic relation with *ETR1* and *CTR1* to be established but it could be either a general negative regulator of the cascade or may be employed in lateral inhibition of hair development in adjacent cells (Galway *et al.* 1994).

Cellular geometry in the root is a central feature of this model. We are proposing differential exposure of epidermal cells to a signal as a result of their position relative to underlying cortical cells. The cells in the cleft overlying the apoplastic space perceive the signal while the other cells do not. Consistent with the role of ethylene in hair cell differentiation is the observation that one member of the ACC synthase gene family is expressed in roots, and that ACC, the ethylene precursor, may move apoplastically. In addition, ACC oxidase activity has been localized to the apoplastic space in tomato (Bradford & Yang 1980; Latché *et al.* 1992; Liang *et al.* 1992; Rodrigues-Pousada *et al.* 1993).

Although this model is indeed naive, it does explain all of our observations both physiological, genetic and structural. It explains in simple terms the effect of differential exposure to a diffusible signal in patterning the root epidermis in *Arabidopsis*. The identification and characterisation of further genes will test the veracity of the model. We shall determine when cells perceive the signal through the use of genetic mosaics. In situ localization of gene products and the exposure of developing roots to pulses of ethylene and ethylene modulators will prove informative. In addition the search for other signals that are involved in the process, most notably negative regulators that may be involved in lateral inhibition will expand our understanding of the development of pattern in this simple system. Of particular interest will be the examination of the role of regulators of epidermal pattern that have been identified in *Arabidopsis*, in other species with dramatically different epidermal organisation.

The authors would like to thanks members of the Root Development Group and Cell Surface Groups within the Cell Biology Department. Research in these laboratories is funded by the BBSRC.

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