seminars in **CELL & DEVELOPMENTAL BIOLOGY,** Vol 9, 1998: pp. 201–206

Root pattern: Shooting in the dark?

*Liam Dolan and Ben Scheres**

Root pattern formation takes place in the embryo and is propagated through subsequent growth and development of the seedling root meristem. Pattern is maintained by positional cues and in some cases by local cell interactions. Such interactions are involved in the balance between cell division and differentiation in cells neighbouring the quiescent centre. This balancing act also occurs in the shoot in which the genetic circuitry underpinning this phenomenon has been characterised. The common genetic mechanism of patterned cell differentiation in the epidermis between the root and shoot extends these parallels further. Given that these shared mechanisms exist, it is tempting to speculate that they reflect the fact that roots may be derived, shoot structures. Alternatively such mechanisms may reflect an evolutionary convergence of genetic mechanism.

Key words: root development / *Arabidopsis thaliana* / intercellular signalling / ethylene / cell differentiation

MERISTEMS ARE SITES of continuous cell proliferation. New cells produced in meristems undergo patterned cell specification during the development of the plant body.[1] To ensure the continuity of this process, a pool of less differentiated stem cells is maintained within the meristem. The processes of cell proliferation and specification are illustrated in their most simple form in the root meristem. Within this meristem, cell files are extended in such a way that they are continuous with pre-existing tissues, leading to the propagation of a simple radial cellular pattern that is initially set down in the embryo. This cellular pattern and the way it is propagated may be described at the cellular level in Arabidopsis[2] (Figures 1 and 2). Therefore, the Arabidopsis root meristem is particularly well suited to answer questions regarding the regulation of the two fundamental cellular processes that occur in meristems: pattern formation and the balance between cell proliferation and cell differentiation. In this review, we will summarise recent findings regarding the mechanism of cell specification in the root epidermis. These studies indicate that similar molecular mechanisms are employed in the regulation of cell fate in the root and the shoot epidermis. Moreover, we discuss the maintenance of stem-cell like properties by a small group of central cells within the root meristem, and possible similarities to the maintenance mechanisms in the shoot meristem.

The earliest vascular land plants, the Rhyniophytes and Cooksonoids were rootless with leafless shoot axes.[3] The first roots evolved among the Lycopsids, a group with its origins in the monophyletic Zosterophylls, which were also rootless.[4] Lycopsid roots were modified lateral appendages, possessing a single leaf trace with exarch xylem differentiation and appear in the fossil record by the early Devonian (380 million years ago). The Trimerophytes constitute a derived sister group to the Zosterophylls, and is the group from which the remainder of vascular plants (Sphenopsida, Pteropsida and seed plants) originated.[3,5] The Trimerophytes were also rootless. Consequently roots evolved first among the Lycopsids and on at least one further occasion during the evolution of vascular land plants. It is nevertheless unclear how many times they arose in the post-Lycopsid lineage.

Position-dependent cell specification in the root epidermis

The Arabidopsis root epidermis is composed of alternating files of two cell types. Trichoblasts (hair forming cells) are located over the anticlinal cortical cell walls and develop root hairs while atrichoblasts overlie the outer periclinal walls of cortical cells and

*From the Department of Cell Biology, John Innes Centre, Norwich NR4 7UH, UK, *Department of Molecular and Cell Biology, University of Utrecht, Padualaan 8, 3584 CH Utrecht, The Netherlands*

1084-9521/98/020201+06 $25.00/0/sr970211

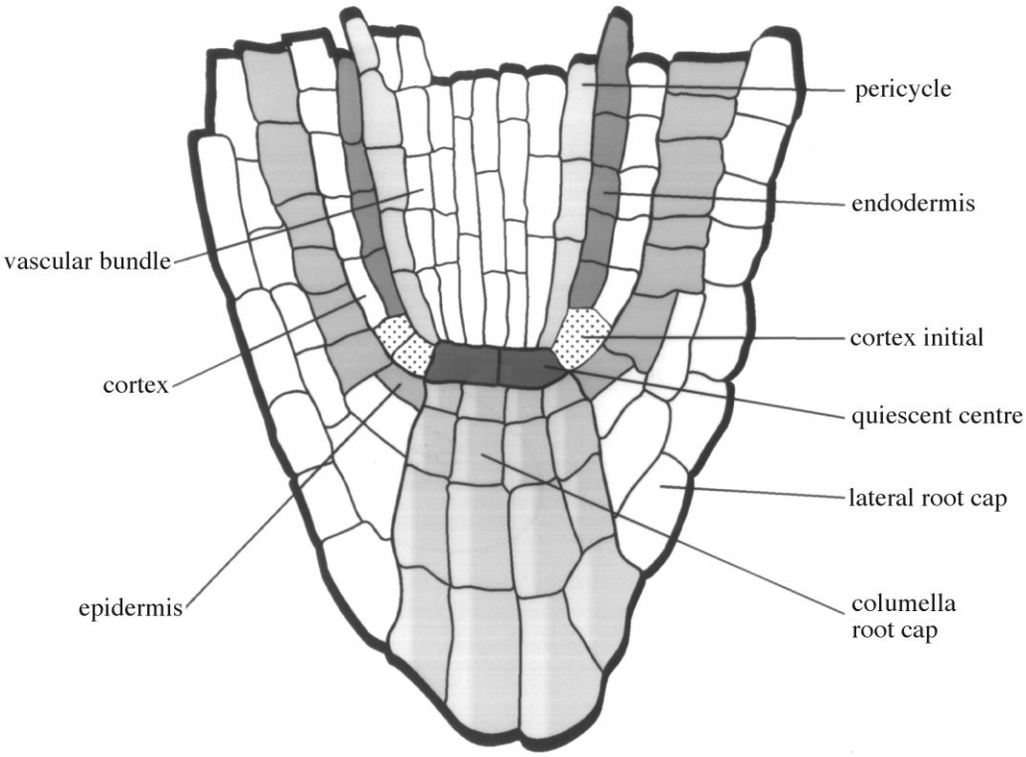

Figure 1. Organisation (longitudinal) of cells in the vicinity of the quiescent centre and initials.

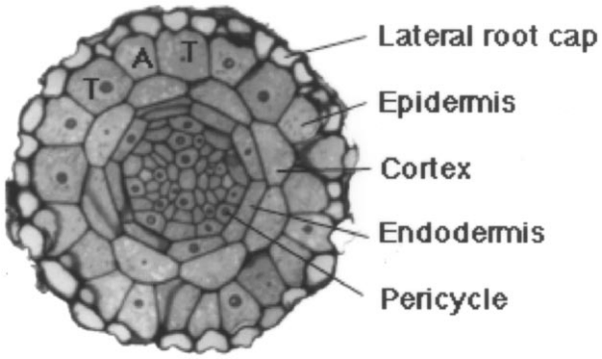

Figure 2. Radial organisation of cells in the root (transverse section of a root made in the meristematic zone). A shows the position of an atrichoblast and T indicates the location of two trichoblasts.

form hairless epidermal cells (Figure 2)[6,7]. The two cell types differ from each other in the meristem in that: (1) trichoblasts divide faster than atrichoblasts; (2) atrichoblasts vacuolate earlier than trichoblasts; and (3) a number of molecular markers are known to be differentially expressed between the two cell types.[6–9] The majority of cell divisions in the future epidermis is transverse, increasing the number of cells per file. Nevertheless, longitudinal anticlinal cell divisions occur at low frequency. Such divisions occur almost exclusively in trichoblasts.[9] They result in a pair of cells, one of which is still located over the anticlinal cortical cell wall, while the other daughter overlies the periclinal cortical cell wall. The daughter cells of such clones assume different fates indicating that positional cues determine their fate. Cell fate is flexible until at least the final cell division in the meristem since two-cell clones are observed in which the cells assume different fates (Berger *et al* unpublished results).

Such position-dependent specification of cell fate has been observed in the epidermis and in other tissues of the root upon laser ablation.[10] It has recently been observed by studying marked clones, generated by heat shock-induced excision of a transposon from a GUS marker.[11] While the majority of clones were restricted to a particular layer, occasionally clones were found which spanned two cell layers and cells developed according to their assumed

position. Both experimental approaches demonstrate that positional cues must be important in the establishment of cell fate in the root. The occurrence of small clones, that by inference are induced higher up the meristem, demonstrates that positional information plays a role throughout the meristem. A similar, flexible mechanism of cell fate specification has been inferred from the analysis of cell clones derived from the shoot meristem.[12] We conclude that despite obvious differences in the reproducibility of cell lineages, root and shoot meristems utilise positional information in a similar way.

Genetic analysis of root epidermal cell pattern

Screening for mutants exhibiting abnormal hair cell patterning in the root epidermis has identified a number of key regulatory genes in the process of patterned cell differentiation. Three genes have been analysed in some detail. *TRANSPARENT TESTA GLABRA* (*TTG*) and *GLABRA2* (*GL2*) are defined by recessive alleles that mutate to a hairy phenotype with all cells in the epidermis forming root hairs.[7,8,13] A further gene, *CAPRICE* (*CPC*), mutates to a decreased hair number phenotype and is defined by a recessive allele containing a T-DNA insert.[14]

GL2 encodes a homeodomain protein which is expressed at low levels throughout the root but at elevated levels in non-hair cells from an early stage in development (near the epidermal/lateral root cap initials).[15,16] It is therefore considered to be a positive regulator of non-hair cell development. Two observations indicate that *TTG* activity is required for *GL2* function. Firstly, the differential rates of cell division observed between the two cell types in wild type and *gl2* are absent from *ttg* mutants.[9] Secondly, *GL2* transcription is lower in a *ttg* background than in wild type roots.

CPC encodes a myb-like protein lacking a transcriptional activator domain suggesting that it may be a transcriptional repressor.[14] Ectopic expression of *CPC* under the control of a CaMV 35S promoter results in the development of hairs on all cells in the root epidermis.[14] This suggests that *CPC* is a negative regulator of the non-hair cell fate. *cpc gl2* double mutant plants exhibit a gl2 phenotype. Therefore, *CPC* may negatively regulate *GL2* in hair cells, thereby positively regulating hair fate, since *GL2* is a positive regulator of non-hair fate.[9] Alternatively, *GL2* may negatively regulate *CPC* activity in non-hair cells, thereby positively regulating the non-hair fate. Analysis of the expression of these genes in mutant backgrounds will resolve this issue.

ttg cpc double mutants exhibit an intermediate (additive) phenotype. There are at least two possible interpretations of this result. Firstly it may mean that both *CPC* and *TTG* act upstream of *GL2*, perhaps at the same point. Alternatively it may mean that while *TTG* acts upstream of *GL2*[13] it does so independently of *CPC*.[14]

Wada *et al* conclude that *CPC* acts as a repressor of *GL2* transcription in hair files.[14] *TTG*, genetically defined as an upstream regulator, may positively regulate *GL2* expression in non-hair cells either directly or indirectly. Other genes that may be involved in this patterning process have been identified but their interaction with *TTG*, *GL2* and *CPC* has not been established. *extra root hair1* (*erh1*), *erh3* and *pom1* have been shown to develop ectopic root hairs and therefore may be considered as either positive regulators of non-hair cell fate or negative regulators of hair fate.[16] Which of these alternatives proves to be true remains to be ascertained.

Shoot epidermal development utilises the same genetic motifs as the root

The above description of the mechanism underpinning the specification of cell fate in the root epidermis highlights the roles played by *TTG*, *CPC* and *GL2*. These genes also play an important role in the establishment of cell fate in the shoot epidermis. Loss of function alleles with glabrous (shoot hairless) phenotypes indicate that *TTG* and *GL2* are positive regulators of trichome (shoot hair) development.[17,18] In addition, *TTG* activity is required for *GL2* action as is the case in the root. *GL1* encodes a *myb*-protein and is required at the same stage as *TTG* in development in the shoot but is not required in the root.[13,19,20] *TRYPTICHON* (*TRY*) mutates to a clustered trichome phenotype but does not exhibit a root phenotype suggesting that it is not involved in the development of the root epidermis.[17] While there is no published *cpc* shoot phenotype, expression of *CPC* under a 35S promoter inhibits trichome formation in the shoot, consistent with its role as a negative regulator in *GL2* transcription.[14] If *CPC* were expressed during normal leaf development it may serve to negatively regulate *GL2* transcription in non-trichome forming cells. It is nevertheless entirely possible that *CPC* is not involved. We await clarification of this issue.

Is the epidermal cell layer exceptional in the use of similar patterning genes in shoot and root-derived tissue? Two genes have been identified as being important players in the establishment of cellular pattern in the root cortex, *SCARECROW* (*SCR*) and *SHORT ROOT* (*SHR*).[21–23] *shr* lacks an endodermis and *scr* roots develop a single layer of questionable identity in the location of endodermis and cortex. The phenotypes of double mutants of each with *fass*, a mutant in which cell proliferation is deregulated, indicate that *SHR* activity is primarily required for the specification of the endodermal tissue layers and *SCR* may have a primary role in asymmetric cell division, although its expression in the endodermis suggests a further role in the stable establishment or maintenance of an endodermal cell layer. It will be interesting to ascertain if these mutations have similar ground tissue defects in the shoot.

Phytohormones act late in root epidermal development: do they have a role in cell specification in the shoot?

Ethylene and auxin are positive regulators of hair cell development.[6,24,25] *CTR1* is a negative regulator of the ethylene response and *ctr1* roots form hairs in the atrichoblast position.[6] Since *CTR1* is a negative regulator of hair cell development in Arabidopsis it indicates that ethylene is a positive regulator. Moreover, *ethylene overproducer3* (*eto3*) roots also produce ectopic root hairs and examination of the phenotypes of *eto1* indicates that hair cells are more sensitive to ethylene than non-hair cells (Cao and Dolan, unpublished). Evidence for a positive role for auxin in the process comes from mutants isolated on the basis of their resistance to elevated exogenous auxin concentrations. For example *auxin resistant3* (*axr3*) and *dwarf* are hairless.[26,27]

Evidence is mounting indicating that ethylene and auxin act independently of *GL2* in the pathway. Firstly, roots harbouring a *GL2* promoter GUS fusion exhibit a normal pattern of expression upon treatment with modulators of ethylene action.[28] This pattern is also unaltered in *ctr1* and *axr2* backgrounds.[28] Treatment of *ttg* and *gl2* plants with blockers of ethylene biosynthesis or perception blocks root hair development.[28,29] Taken together, these observations indicate that the hormones act independently of *TTG* and *GL2* function and differential ethylene sensitivity of epidermal cells is independent of *TTG* and *GL2* function. The time at which hormones act in this process suggests that they may be the mediators of environmental responses given that environmental factors can have profound effects on the development of cell differentiation in the root epidermis.[29]

To date no clear role has been demonstrated for the phytohormones in the specification of cell type in the shoot epidermis. A key development in this area is the recent illustration of the effect of environmental factors on guard cell development in Arabidopsis. Serna and Fenoll[30] showed that altering the growth conditions of plants can lead to the specification of additional guard cells. Since environment-induced alteration in cell fate in the root may be mediated by phytohormones, it is possible that the changes in guard cell fate described by these authors might involve the same suite of molecules.

The balance between cell proliferation and differentiation

A balance is maintained in the root meristem between the progression of cell differentiation, ultimately producing terminally differentiated cells, and the maintenance of a pool of cells that can divide in an indeterminate way to generate new cells. All cell files terminate with their respective inititals in the Arabidopsis root (Figure 1)[2]. Initial cells display distinct cell division and cell differentiation characteristics, which distinguishes them from their daughters. For example, columella initial cells divide whilst their daughters cannot, and the daughters contain characteristic starch granules not present in the initial. The epidermal initial undergoes a periclinal division that generates a new lateral root cap layer, and its daughters do not. In a similar fashion, the cortical initial (in the majority of ecotypes) first divides to generate a daughter, which subsequently undergoes a periclinal division to generate two separate layers with distinct identity: cortex and endodermis. What keeps the initials in this relatively less differentiated state? A clue is provided by the observation that the initials in Arabidopsis contact four central cells (quiescent centre, QC) which are mitotically less active. Recently it has been demonstrated that laser ablation of single quiescent centre cells leads to the progression of differentiation status of initial cells, which become similar to their daughters in the expression of differentiation markers or in their cell division pattern.[31] The QC-dependent activity governing this effect acts over a single cell distance, possibly in a contact-dependent manner, as neighbouring QC cells cannot

rescue the progression in differentiation of initial cells that flank a dead QC cell. Evidence that QC-mediated control of cell differentiation is independent of cell division control comes from the observation that the inhibition of progression of cell differentiation of initials by the QC is also exerted in mutants that display no post-embryonic cell division in the root meristem.[11]

The existence of a slowly dividing region in the root meristem that controls the balance between cell differentiation and cell proliferation is strikingly similar to the situation in the shoot. In the shoot the *CLV* genes are involved in controlling this balance as *clv1* mutants display over proliferation of the shoot meristem.[32–34] *CLV1* encodes an LRR-receptor kinase and is expressed in the central zone of the SAM, with some overlap in the peripheral regions destined to form leaf primordia.[35] Interestingly, homologues of *CLV1* have recently been identified which are specifically expressed in the root meristem (Heidstra and Scheres, unpublished data). It will be interesting to find out whether the function of these root specific *clavata1-like* genes is similar to *CLV1* function in the shoot. In any case a functional similarity between the central zone of the shoot meristem and the quiescent centre of the root meristem appears to exist.

Conclusions

Recent analyses of root development in Arabidopsis have revealed three novel insights. First, despite regular cell lineages, cell fates are regulated by positional signalling. Second, cell fate of epidermal cells is regulated by a module containing among others the *TTG* and *GL2* genes. Third, a central zone of cells, the quiescent centre, is involved in the control of cell differentiation status of neighbouring cells. Mechanisms of all three processes are reminiscent of corresponding processes in the shoot.

Simplistically, there are two possible explanations for our observations. Firstly it is possible that roots may be derived from ancestral shoot structures. If this were correct, we might anticipate that a number of key genetic changes occurred during the transformation of an ancestral shoot-like organ to the relatively derived root. A consequence of such a process would be the utilisation of similar molecules (gene products) in the regulation of meristem development and function in both organ systems. We suggest here that such a conservation has been demonstrated in the case of epidermal cell specification. Alternatively, it is possible that the suite of genes required for the specification of cell fate in the shoot epidermis of these ancient (ancestral) plants was coopted into a similar role upon evolution of the root (from a structure other than the root meristem, perhaps a modified lateral appendage which is the case in the Lycopod lineage). Over time, some of the elements may have been lost (e.g. *GL1* and *TRY* have no apparent function in roots) or others may have been incorporated. Now that many of these genes have been cloned it is possible to test these hypotheses in roots and shoots from diverse groups of plants. It is plausible that the same is true for the regulation of proliferation versus differentiation in the two meristem types.

The finding that there exist similarities in the molecular mechanism underpinning cellular patterning and proliferation versus differentiation in the shoot and root of higher plants has obvious implications for our understanding of the process of organ evolution in seed plants. The extension of these studies to non-seed plants will be required to determine if similar molecules were coopted independently numerous times over the last 400 million years to generate similar though non-homologous organ systems.

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