Root development: new meanings for root canals?

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During *Arabidopsis* root development, a radial pattern of tissues is extended by the meristem. These tissues form continuous layers and recent data suggest that tissue continuity is instrumental for constraining the direction of signaling in a process termed channeling. In the ground tissue, fate-determining signals originate from contiguous cells of the same layer, possibly due to specific symplastic connections. Mutant analysis supports the hypothesis that vascular tissue continuity may facilitate and depend on the directional transport of a vascular fate-determining signal, possibly the phytohormone auxin.

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Abbreviations

IAA	indole-3-acetic acid
mp	*monopteros*
pin	*pin-formed*
scr	*scarecrow*

Introduction

Despite tremendous differences in morphology, all plant organs are composed of a limited number of different cell types, which are arranged in a basic radial pattern to form continuous tissue layers. Prototypic versions of this basic radial pattern are laid down in the embryo. During post-embryonic development, existing organs are extended and new organs are formed, while tissue continuity is preserved. Distal groups of mitotically active cells, the root and shoot meristems, play a major role in this process [1]. Many clonal analyses have been carried out and they revealed that strict relationships between differentiated cells and their progenitors in the shoot apical meristem are in general absent [2,3]. This led to the immediate realization that cell-to-cell communication must be an important mechanism in pattern formation of differentiated cells. The relative importance of signaling events within the meristems, and signaling between meristems and preexisting tissues remained unknown. The *Arabidopsis* root, however, has recently turned out to be an appropriate system to address these issues because of its regular structure.

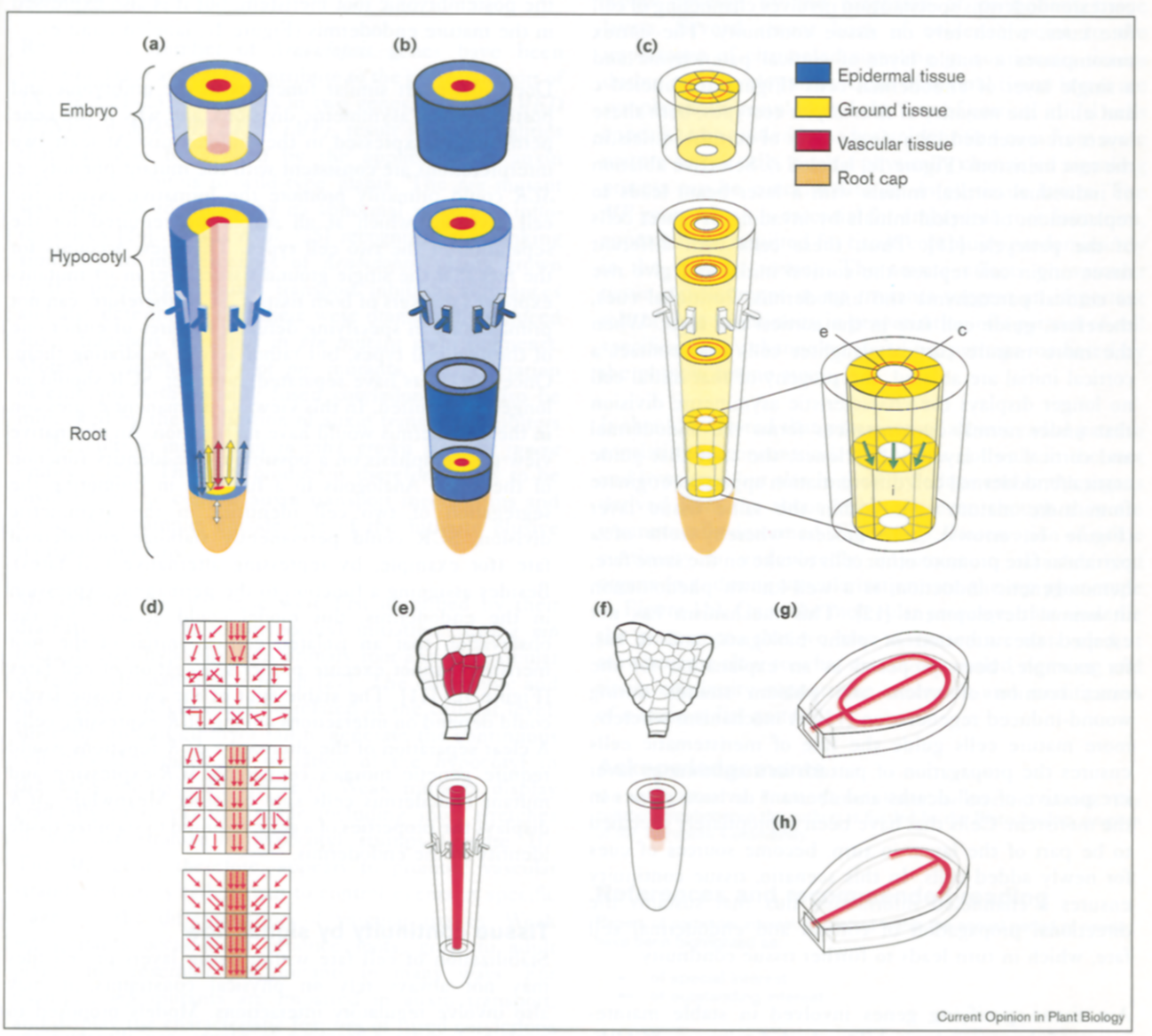
Root meristems lack lateral organ formation, a process that complicates the analysis of cellular patterning in the shoot apex. Roots are cylindrical structures with a radial pattern of concentrically arranged cell layers of three main tissues: epidermis, cortical (ground) tissue, and stele tissue (Figure 1). The root of the crucifer *Arabidopsis thaliana* contains a simple and almost invariant cellular pattern, with single layers of different cell types surrounding a small central vascular bundle [4]. The distally located root meristem perpetuates this radial pattern by regular cell divisions to give rise to continuous tissue layers (Figure 1a). The hypocotyl that connects the seedling root to the remainder of the plant has a similar tissue arrangement, and like its root counterpart this tissue pattern originates from the separation of cell layers in the early embryonic axis (Figure 1a) [5,6]. The root meristem can therefore be considered as a particularly clear and geometrically simple example of maintenance of a basic radial pattern, consisting of concentric tissue layers, that is laid down early during embryogenesis.

Both surgical and genetic studies on root development are beginning to reveal a common theme—channeled signaling—which is the focus of this review. The term canalization has been utilized in a model for vascular strand formation where signal flow is restricted by regulatory interaction (Figure 1d) [7]. Here, we define channeling in a broader sense as the restriction of any signaling path either as a result of regulatory interactions, or due to physical constrictions (for example routing via plasmodesmata). We will argue that signaling activities involved in cell fate decisions in the root act along constrained paths of physical or regulatory nature, thereby perpetuating a pre-pattern of cell specification. To form and maintain these restricted paths, tissue continuity appears to be important.

Perpetuation of the radial pattern in the root and channeled specification cues?

Studies on the connectivity of individual cells in the root indicate that the anatomical continuity of tissue layers correlates with cytoplasmic continuity. Micro-injection and loading experiments with fluorescent dyes in different regions of *Arabidopsis* root tips have revealed specific patterns of dye-coupling, that is, the ability of dyes to move between neighbouring cells via plasmodesmata. Plasmodesmatal connections between epidermal cells appear to be tissue-specific at particular differentiation stages. Incipient root epidermal cells are dye-coupled to all neighbours in the root meristem, reminiscent of the complete symplastic coupling in the embryo [8], but they remain coupled exclusively within their own tissue later on, to end up isolated upon further differentiation ([9]; Figure 1b, thick lines outline coupled domains). Fluo-

Figure 1



Plant development as an extension of a basic radial pattern. **(a–c)** Tissue layers in wild-type embryo (upper part), seedling hypocotyl (lower part, above root hairs), and seedling root (lower part, below root hairs). **(d–h)** Hypothetical signal channeling to promote vascular development. (a) Cell division activity (arrows) in the meristem extends the basic radial pattern. The meristem also adds new cells to the distal-most region of the root to replenish root cap cells, a feature that is not discussed here. (b) Symplastic connection of epidermal tissue. Solid black lines represent symplastic connection of epidermal cells in the embryo and in seedling regions. Differentiated root hairs are isolated, epidermal cells in the elongation zone are connected within their own tissue, and embryonic as well as meristematic epidermal cells are completely connected [8–10]. (c) Separation of layers in the ground tissue, the red line separating divisions. Signals (green arrows) from more mature cortical cells are required to reinforce cell fate of initial cells [11]. e, endodermis, c, cortical parenchyma, i, initial. (d) Canalization model: a regulatory feed-back mechanism promotes differentiation of signal transducing cells, such that their conductivity is further enhanced. The mechanism results in differentiation along continuous lines. (Modified from [7]). (e–h) The vascular system in *mp* mutants and wild-type plants: the mutant vascular system (f,h) is incomplete and discontinuous compared to corresponding wild-type structures (e,g). Individual cells do not show marked abnormalities, yet tissue continuity is severely affected particularly in the terminal regions of hypocotyl (f) and cotyledon (h). This distribution of defects is suggestive of insufficient induction from a vascular differentiation promoting signal flow.

rescent dyes also indicate the existence of tissue-specific coupled domains in the cortical parenchyma [10]. The emerging picture is one of continuous tissue layers, in which plasmodesmata form physically constrained paths in the vicinity of the meristem where cell identity needs to be reinforced.

Surgical studies in *Arabidopsis* roots have revealed that cortex/endodermis specification involves channeling of cell fate cues, which rely on tissue continuity. The cortex encompasses a single layer of cortical parenchyma and a single layer of endodermal cells (Figure 1c, labeled c and e). In the majority of *Arabidopsis* ecotypes, both these layers are extended by a single layer of cortical initials in the root meristem (Figure 1c, labeled i). Selective ablation of individual cortical initials with a laser beam leads to replacement of cortical initials by invading daughter cells of the pericycle [11]. Thus, these cells with different tissue origin can replace the cortical initial and give rise to cortical parenchyma and endodermis. Positional cues, therefore, guide cell fate in the cortical cell layer. When the more mature cortical daughter cells that contact a cortical initial are ablated, the progeny of that initial cell no longer displays the characteristic asymmetric division that under normal circumstances forms the endodermal and cortical cell layers [11]. Hence, the cues that guide cortical/endodermal cell differentiation appear to originate from more mature cells within the same tissue layer (Figure 1c, arrows). The process whereby cells of a particular fate promote other cells to take on the same fate, homoiogenetic induction, is a well-known phenomenon in animal development [12]. This mechanism has not escaped the attention of plant biologists, and it has, for example, been proposed as an explanation for the exact reunion of xylem and phloem strands during wound-induced regeneration [13]. A mechanism whereby more mature cells guide the fate of meristematic cells ensures the propagation of pattern in a continuous layer irrespective of cell deaths and aberrant division planes in the meristem. Cells that have been appropriately specified to be part of the layer, in turn, become sources of cues for newly added cells. In this scenario, tissue continuity ensures a channeled information flux that sustains the directional propagation of cortical and endodermal cell fate, which in turn leads to further tissue continuity.

Are tissue-specifying genes involved in stable maintenance of tissue continuity? The *Arabidopsis* gene *SCARECROW (SCR)* is required for the formation of distinct endodermis and cortex layers. In the embryo, the subdivision of a common ground-tissue layer into two distinct layers occurs by tangential divisions within a ring of cortical cells at heart stage (Figure 1c, upper panel; for a more detailed description of radial pattern formation in the embryo, see [14]). The two newly formed tissue layers are then stably maintained by the activity of the primary root meristem. The elongation process involves the permanent recapitulation of the separating asymmetric divisions in the postembryonic root meristem (Figure 1c). In *scr* mutants, these tangential divisions in the ground tissue layer do not occur, neither in the embryo nor in the postembryonic root meristem [15] and the single ground tissue layer expresses both cortex and endodermis markers [16••]. The *SCR* gene has been isolated and encodes a putative transcriptional regulator [16••]. *SCR* is expressed in the asymmetrically dividing ground tissue precursor cells in the embryo and in the postembryonic root meristem, but it is also expressed in the mature endodermis (Figure 1c, labeled i and e).

Does *SCR* exert similar functions in the embryonic and postembryonic asymmetric divisions, and why is the gene permanently expressed in the endodermis? At least two interpretations are consistent with the mutant phenotype. *SCR* could primarily promote the formative asymmetric cell divisions which at all stages are required for the separation of the two cell types. This view accounts for the fact that the single ground tissue layer in *scr* mutants expresses markers of both tissues. SCR, therefore, can not primarily act in specifying defining features of either one of the two cell types, but rather acts in separating them. Once the layers have separated, however, SCR should no longer be required. In this view, its permanent expression in the endodermis would have no function. An alternative view puts emphasis on a possible tissue-identity function of the gene. Analogous to a function in promoting the segregation of two cell identities in the asymmetric division, SCR could permanently stabilize endodermal fate (for example, by repressing alternative cell fates). Besides assigning a function to the permanent expression in the endodermis, this model could account for the observation that an isolated cortical initial of the root meristem cannot execute proper segregation of cell fates (Figure 1c) [11]. The stable split of the two tissue layers could depend on interactions among *SCR*-expressing cells. A clear separation of the alternative *SCR* functions would require genetic mosaics containing *SCR*-expressing and mutant endodermis cells side by side. Meanwhile, *SCR* displays the properties of a gene required to stabilize tissue identity in the endodermis.

Tissue continuity by signal flow

Stabilization of cell fate within tissue layers or cell files may not always rely on physical constraints; it may also involve regulatory interactions. Models proposed to explain vascular patterning in plants, can illustrate this point. Vascular tissues form ramified systems of continuous cell files each made of elongated, interconnected cells. Continuity is crucial for vascular function and different models of vascular strand formation have to account for the fact that recruitment of cells for vascular differentiation occurs along continuous lines [17,18]. This one-dimensional localized differentiation could reflect the channeling of a signal molecule. A simple mechanism to account for this process is a positive feedback rendering conducting provascular cells even more conductive, which has been termed canalization (Figure 1d) [7,18]. IAA (indole-3-acetic acid, the major form of auxin in higher plants) is a suspected signal molecule involved in vascular differentiation. IAA is known to be transported in the basal direction [19]. Moreover, an IAA source will induce a new vascular strand extending basally from the position of the source [7]. In addition, auxin transport has been implicated

in a number of developmental processes, including root initiation.

Recently, a number of *Arabidopsis* genes have been identified that appear to contribute to the polar transport of IAA [20•,21–23]. Mutations in two genes, *PIN FORMED (PIN)* and *MONOPTEROS (MP)*, result in abnormalities that can also be evoked by the application of auxin transport inhibitors to wild-type plants. The *pin* mutant was originally identified by its abnormal spike-like inflorescences [24]. Okada *et al.* [25] recognized the striking similarity to inflorescences of *Arabidopsis* plants grown in the presence of auxin transport inhibitor substances and also determined that there were dramatically reduced auxin transport capacities in *pin* mutant stem segments. Moreover, another trait of *pin* mutants, the generation of embryos with variably fused cotyledons, can also be mimicked by the application of auxin transport inhibitors to the culture medium of *in vitro* grown *Brassica juncea* embryos [26]. The PIN product is therefore likely to be involved in the cellular auxin transport mechanisms and could, for example, encode one of the hitherto elusive transport proteins.

Mutants for the *MP* gene were first identified by their dramatic effect on root/hypocotyl initiation. Strong *mp* mutants lack hypocotyl and primary root and the vascular system is dramatically reduced [27]. The embryonic and the vascular defect could be related at the cellular level, since *mp* mutant embryos fail to generate the continuous cell files that mark the initiation of the hypocotyl in the early embryo (Figure 1e,f). Weak mutants display gradually more vascular tissue including short stretches of vascular strands in hypocotyl stumps (Figure 1f). The *MP* gene, therefore, appears to promote vascular strand continuity rather than to control events at specific stages in the differentiation of vascular tissues. Weak *mp* mutants occasionally produce roots, grow to adult plants and resemble *pin* mutants in many ways. For example, *mp* mutants are impaired in auxin transport, and display the characteristic features of fused cotyledons and spike-shaped inflorescences [28••]. It has, therefore, been proposed that the *MP* gene has a primary function in relaying auxin related apical-basal signals required for oriented cell differentiation of the vascular system [28••]. From the perspective of the channeling concept, *MP* could have a general role in mediating several types of developmental responses to auxin flow, such as vascularization and root initiation, while the action of pin might be restricted to certain organs and stages. Interestingly, both the *PIN* gene and the *MP* gene have recently been isolated and encode a putative membrane protein (K Palme, personal communication) and a putative transcriptional regulator (C Hardtke, T Berleth, unpublished data) respectively. It is tempting, therefore, to speculate that PIN could mediate directional auxin transport, while MP could be involved in relaying this signal to ensure appropriate differentiation of cells that receive it.

Conclusions

Roots develop by the extension of continuous tissue layers. Several recent lines of evidence point to the importance of channeled signaling in continuous tissues during root development. Cells of several tissue layers are symplastically coupled and the fate of cortical initial cells appears to be guided by as yet unidentified cues from more mature cortex cells. Such a physically channeled signaling process can be conceived to act in all tissue layers. The *MP* gene, which is essential for embryonic root formation, appears to be required for tissue continuity predominantly in the vascular tissues. The severe effects on vascular tissue development in *mp* mutants suggest an important role of tissue continuity in perpetuation of a pattern of cell specification by channeled signaling, perhaps mediated by the phytohormone auxin.

It should be noted that extension of a basic radial pattern consisting of concentric layers occurs not only in the root and hypocotyl but also in the cotyledons and in the organs derived from the shoot meristem, even though they are geometrically deformed (Figure 1g). Preservation of tissue continuity is undoubtedly important in shoot-derived organs (e.g. during vascularization; see the *mp* phenotype in Figure 1h). Once the molecular processes that underlie the phenomena that we have discussed are unraveled, it may become clear to what extent channeled signaling and tissue continuity support the extension and modification of a basic radial pattern.

Acknowledgements

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