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How many ways are there to make a root?

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Plants often make the same organ in different development contexts. Roots are a quintessential example, with embryonic, primary, lateral, adventitious, and regenerative roots common to many plants. The cellular origins and early morphologies of different roots can vary greatly, but the adult structures can be remarkably similar. Recent studies have highlighted the diversity of mechanisms that can initiate roots while late patterning mechanisms are frequently shared. In the middle stages when patterning emerges, evidence shows that antagonistic auxin–cytokinin interactions regulate tissue patterns in root embryogenesis, vascular organization, and regeneration but it is not yet clear if a common ontogeny for the root body plan exists.

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The same organ from diverse origins

The plant has the ability to not only grow throughout its adult life but also, as a part of normal development, produce entirely new organs not present in the embryo [1,2]. The root is an interesting example because the origins of new roots can be highly diverse. For example, in the *Arabidopsis* embryo, the first step of root formation occurs at the early globular stage when a single cell — the hypophysis — is recruited by the embryo proper [3]. The hypophysis eventually generates two of the unique cell types and tissues of the root — the quiescent center (QC) and the columella — while other cells of the embryo proper are recruited to form lateral root cap and the rudimentary radial files [4].

Lateral roots, for which the mature morphology is identical to the primary root in *Arabidopsis*, could not have a more different origin. In the adult plant, a small group of cells in a single radial file many daughters removed from the stem cells — the pericycle's lateral root founder cells — divide in a stereotypical manner to produce the entire lateral root [5]. Unlike the predictable origins of tissues in the embryo, division patterns are more stochastic in the early lateral root formation, such that cells do not appear to be fated to give rise to specific tissues at early stages [6•]. In yet another instance of adult root formation, adventitious roots arise from a variety of different vasculature-associated cell types in the adult plant, although early stages of their ontogeny are not yet well described [7].

The plant can also make roots spontaneously after damage or excision of existing roots in a regenerative process [8]. It was shown that *Arabidopsis* roots that regenerated via auxin-induced callus — a proliferation of highly potent cells — actually derive from pericycle cells and follow a lateral root program [9•,10•,11]. Interestingly, the pericycle layer is competent to regenerate organs in many parts of the plant but goes through a root development program even when generating shoots [9•]. The key meristem regulators — *PLETHORA3* (*PLT3*), *PLT5*, and *PLT7* — appear to have a redundant role in multiple steps inducing root meristem regulators and then, if areal organs are induced, shoot developmental genes [12•]. Given that callus forms naturally during regeneration in many plant species, the lateral root program may be a common path to regeneration.

However, the plant also appears to have more than one developmental path to root regeneration. In a recent example, roots that regenerated from cut leaves without exogenous hormone treatments appeared to arise from procambium and follow an adventitious root program [13••]. In addition, the root meristem can recover from severe damage using mechanisms that do not resemble either lateral or adventitious root formation [14••,15•]. Overall, the plant shows truly diverse origins of roots, setting up an interesting question about re-use vs. re-invention of the mechanisms that shape its highly conserved body plan. Experimentally, the developmental diversity of root origins amounts to a rare comparative system that makes use of the same genome.

Late stage convergence

To address last steps first, it is clear that many of the same genes function in patterning at stages when the morphology of different roots begins to converge [1]; for example, in the well-studied GRAS family transcription factors, SHORT-ROOT (SHR) protein was shown to move from the stele into the outer neighboring layer to contribute to

endodermal identity and lead to a formative division of the ground tissue through *SCARECROW* (*SCR*) [16,17,18]. Mutants in these transcription factors lead to similar patterning and cellular identity defects in the embryo [19], primary roots [18,19], lateral roots [20•], and regenerative roots [15•]. However, interestingly, even *SHR*, whose function in primary roots is conserved in monocots [21], does not appear necessary for patterning in areal-borne anchor roots, at least in the dicot model *Arabidopsis* [20•]. This may serve as a warning not to assume re-iteration of even highly conserved core programs.

Nonetheless, many mechanisms that operate in the late stage mature root are conserved in other types of roots [22–25]. This review will focus on earlier stages of root development when the anatomy of the early root can be vastly different but a core body plan — stele, ground tissue, epidermis, and a root cap — takes shape.

Common and divergent cues for initiation

The phytohormone auxin is a necessary and common signal in all instances of root formation examined so far [7,26–28], and more specifics on auxin will follow. However, a body of work has now shown how divergent early signaling mechanisms can be.

For example, mutants in *ABERRANT LATERAL ROOT FORMATION4* (*ALF4*) have a normal primary and embryonic root but are defective in the formation of lateral roots, adventitious root formation, and regenerative roots from callus but not regeneration of primary root [9•,13••,14••]. *AUXIN RESPONSE FACTOR 7* (*ARF7*) and *ARF19* together are defective in lateral root formation but not callus derived or adventitious root formation [13••]. Some of the mechanistic specificity of different root development programs appears to be due to the fact that paralogs within gene families have taken on organ-specific expression patterns and functions. For example, gain-of-function mutations in different members of the family of *Aux/IAA* auxin signaling inhibitors block embryogenesis (*bodenlos* [29]) or lateral development (*solitary root* [30]).

Nonetheless, there are cases that strongly implicate independent signaling pathways in the regulation of different root developmental programs. For example, one recent study showed that *WUSCHEL-RELATED HOMEOBOX11* (*WOX11*) and *WOX12* were required for adventitious root formation from excised leaves and for callus formation but not for lateral root formation [13••]. It was shown, through SRDX repressor domain fusions, that *WOX11/12* were needed for induction of *LATERAL ORGAN BOUNDARIES DOMAIN16* (*LBD16*) and *LBD29* in adventitious roots [13••], while it was previously shown that *ARF7* and *19 directly* regulated the *LBD*s in lateral roots [31]. This led the authors to speculate that an independent mechanism was needed for adventitious root formation from excised leaves, perhaps because its position and timing is more responsive to environmental cues.

In lateral root development, several groups have shown the importance of the mechanical role of the endodermis as a signaling mechanism for lateral root initiation. For example, it was shown that blocking auxin responses specifically in the endodermis prevented morphological changes within the cell that then affected lateral root outgrowth non-cell autonomously in the adjacent pericycle [32••]. Interestingly, the changes in endodermis blocked lateral root initiation at its earliest stage, suggesting a role for mechanical signals at the organ initiation stage [32••]. This mechanical signaling role of the endodermis was reinforced by another report showing that ablation of endodermal cells alone can lead to the first step of lateral root initiation by inducing division of the pericycle [33••]. In agreement, ablation appeared to carry out the role served by auxin response, which regulated cell wall loosening. In addition, auxin was needed just internally, in the pericycle, to promote the proper orientation of cell division in the growing lateral root [33••]. The morphology underlying this signaling environment is so specific to roots born from internal radial files that it is hard to imagine an analogous signaling system in place for root initiation in the embryo or during regeneration, although further exploration of the role of mechanical forces in root initiation will be interesting.

Patterning by auxin–cytokinin antagonism

Progress on the mechanisms that underlie the cross-talk between auxin and cytokinin have answered some long-standing questions about how these two hormones antagonistically interact to shape the root body plan [34]. For example, in *Arabidopsis*, the vascular cylinder is initially radially symmetrical in the embryo before xylem and phloem pole appear [35]. A feedback loop is set up in which cytokinin signaling in the outer flanks orients the *PIN-FORMED1* (*PIN1*) auxin efflux carrier toward a midline. The build-up of auxin induces the negative cytokinin signaling regulator *ARABIDOPSIS HISTIDINE PHOSPHOTRANSFER PROTEIN 6* (*AHP6*), which makes the region 'blind' to cytokinin signaling and specifies the position of xylem [36••]. The early symmetry of the vasculature appears to be broken initially by the spread of *AHP6* from the bisymmetrical cotyledons at heart stage [36••,37]. The feedback between auxin, cytokinin, and cell identity mechanisms has been modeled as a self-organizing system that could explain the patterning of xylem and phloem in the vasculature [38••].

In the adult meristem, prior work in the adult root has shown that an intersection of high auxin concentration