Cell, Vol. 119, 109–120, October 1, 2004, Copyright ©2004 by Cell Press

The *PLETHORA* Genes Mediate Patterning of the *Arabidopsis* Root Stem Cell Niche

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Summary

A small organizing center, the quiescent center (QC), maintains stem cells in the *Arabidopsis* root and defines the stem cell niche. The phytohormone auxin influences the position of this niche by an unknown mechanism. Here, we identify the *PLETHORA1* (*PLT1*) and *PLT2* genes encoding AP2 class putative transcription factors, which are essential for QC specification and stem cell activity. The *PLT* genes are transcribed in response to auxin accumulation and are dependent on auxin response transcription factors. Distal *PLT* transcript accumulation creates an overlap with the radial expression domains of SHORT-ROOT and SCARECROW, providing positional information for the stem cell niche. Furthermore, the *PLT* genes are activated in the basal embryo region that gives rise to hypocotyl, root, and root stem cells and, when ectopically expressed, transform apical regions to these identities. Thus, the *PLT* genes are key effectors for establishment of the stem cell niche during embryonic pattern formation.

Introduction

Embryogenesis in higher plants produces a seedling with two distally localized stem cell groups at opposite ends. During postembryonic development, these stem cells continuously provide cells to maintain two mitotic cell populations, the shoot apical meristem (SAM) at the apical end and the root meristem (RM) at the basal end (Weigel and Jürgens, 2002; Laux, 2003). A pivotal issue in plant embryogenesis is how the stem cell sets that give rise to shoots and roots are specified at opposite poles to define the sites for postembryonic development.

Both shoot and root stem cells in *Arabidopsis thaliana* are maintained by the activity of nearby "organizing" cells, reminiscent of the dependence of animal stem cells on local cellular environments called stem cell niches (Spradling et al., 2001). In the SAM, generation and maintenance of stem cells requires an underlying organizing center, which is specified by the *WUSCHEL* (*WUS*) homeobox gene (Mayer et al., 1998). *WUS* is first expressed in four inner cells of 16-cell-stage embryo, but it is unknown how the position of the *WUS* expression domain is determined.

In the RM, all stem cells surround the mitotically less active quiescent center (QC). The QC forms an organizing center which is required for stem cell maintenance (van den Berg et al., 1997; Figure 1A). *SHORT-ROOT* (*SHR*) and *SCARECROW* (*SCR*), which encode members of the GRAS family of putative transcription factors, are required for QC identity in addition to their role in radial patterning (Di Laurenzio et al., 1996; Helariutta et al., 2000; Wysocka-Diller et al., 2000; Sabatini et al., 2003). *SHR* is transcribed exclusively in the provascular tissue from embryogenesis onward, but the protein moves outwards to the surrounding cell layers including the QC and promotes *SCR* expression in these cells (Nakajima et al., 2001). *SCR* is cell-autonomously required for QC identity, which in turn promotes the activity of surrounding stem cells (Sabatini et al., 2003). As the SHR/SCR pathway specifies the entire layer surrounding provascular tissues in the root, it is necessary but not sufficient to define the exact position of the stem cell niche.

The phytohormone auxin influences the location of the stem cell niche. From embryogenesis onward, an auxin response maximum is formed and maintained in the distal stem cell region (Sabatini et al., 1999; Friml et al., 2003b). Auxin response or transport mutants display root patterning defects and exogenous application of auxin induces ectopic QC and stem cells (Sabatini et al., 1999; Friml et al., 2002). Auxin has also been linked to the formation of the entire embryonic root. For example, the *MONOPTEROS* (*MP*) gene encodes a member of the auxin response factors (ARFs) that can bind to promoter elements of auxin-inducible genes and is required for embryonic root formation (Berleth and Jürgens, 1993; Hardtke and Berleth, 1998; Ulmasov et al., 1999). Several other genes involved in auxin signaling have also been implicated in this process (Hamann et al., 1999, 2002; Hobbie et al., 2000; Hellmann et al., 2003). Thus, auxin may act as a cue for specification of the embryonic root as well as for the postembryonic stem cell niche. However, transcription factors that act downstream of auxin in patterned organ and cell type specification have not been identified.

Here, we describe the *PLETHORA1* (*PLT1*) and *PLT2* genes, encoding AP2 type putative transcription factors first expressed in the basal embryo region, then in the embryonic root primordium, and later in the RM stem cell niche. These genes are required for stem cell specification and maintenance in the RM and act in parallel

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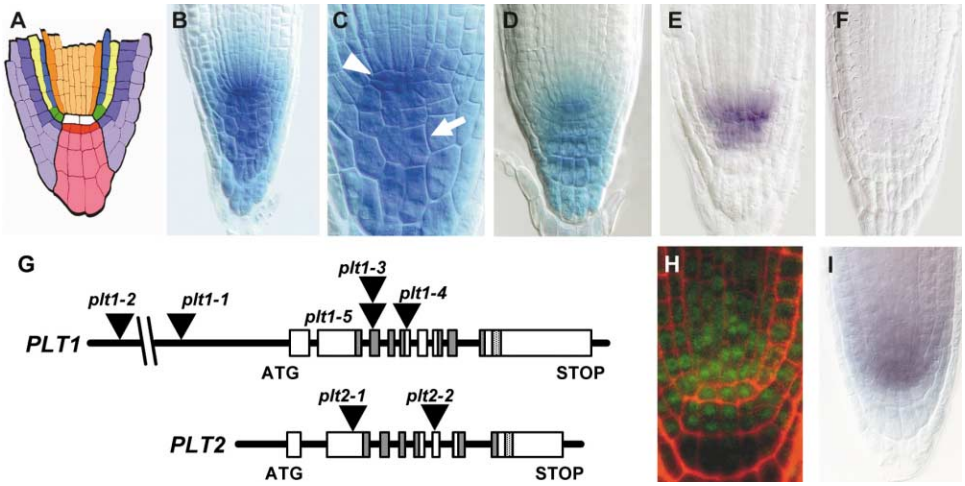

Figure 1. *PLT1* and *PLT2* Encode AP2-like Putative Transcription Factors Expressed in the Distal Root Meristem

(A) Median longitudinal view of the *Arabidopsis* root meristem. White, QC; red, columella stem cells; pink, columella root cap; light purple, lateral root cap; purple, epidermis; green, cortical/endodermal stem cells; yellow, cortex; blue, endodermis; orange, pericycle; light orange, provascular cells.

(B–D) GUS staining of the COL148 promoter trap line (*plt1-1*) at 4 days postgermination (dpg). (B) Root from homozygous plants. (C) Magnification of (B); arrowhead, extra QC cells; arrow, extra columella cells. (D) Root from heterozygous plants.

(E and F) Whole-mount in situ hybridization with *PLT1* probe in wild-type WS (E) and *plt1-1* (F) roots at 4 dpg.

(G) Genomic structure of *PLT1* and *PLT2*. Boxes indicate coding sequence. Gray boxes, AP2 domains; dotted boxes, putative nuclear localization signal; triangles, insertion sites in *plt* mutants.

(H) GFP-PLT1 fusion protein driven by the *PLT1* promoter.

(I) Whole-mount in situ hybridization with *PLT2* probe in WS.

with *SCR*- and *SHR*-mediated patterning input to define QC and stem cell position. Transcription of the *PLT* genes is stimulated by auxin and is dependent on ARF transcription factors. Ectopic *PLT* expression in the embryo induces homeotic transformation of apical domains into root stem cells, roots, or hypocotyls, showing that *PLT* genes are major determinants for the root stem cell niche and indicating a wider role in basal embryo identity.

Results

The *PLETHORA* Genes Encode Putative Transcription Factors of the AP2/EREBP Class Specifically Expressed in the Root Stem Cell Region

Candidate genes encoding transcription factors that specify root stem cells have not emerged from forward genetic screens. We therefore adapted a promoter trap strategy to screen for transcription factors specifically expressed in the distal root. We screened expression patterns in ~15,000 T-DNA insertion lines harboring a promoterless β-glucuronidase (GUS) gene adjacent to the right border of the T-DNA (Bechtold et al., 1993). We identified two lines (COL148 and QC3) in which GUS staining was observed exclusively in the distal region of the RM. COL148 showed staining in a relatively broad region including QC, surrounding stem cells, and columella root cap cells (Figure 1B). In QC3, weaker staining was observed mainly in the QC and surrounding stem cells (data not shown). The COL148 line carries a recessive mutation that causes production of extra cells in columella root cap and the QC (Figure 1C). The same phenotype occurs in the QC3 line, although with a lower penetrance. Complementation tests showed that the two lines carry allelic mutations. Based on the excess of columella and QC cells, we designated the mutations in COL148 and QC3 as *plethora1-1* (*plt1-1*) and *plt1-2*, respectively.

The *plt1-1* and *plt1-2* alleles carry T-DNA insertions in the promoter region of the At3g20840 gene at 1.2 and 5.4 kb upstream of the predicted coding region, respectively (Figure 1G). The insertion in *plt1-1* cosegregates with the mutant phenotype (see Experimental Procedures), indicating that the T-DNA insertion causes the phenotype of this allele. Furthermore, three independent insertion alleles within the At3g20840 coding region display essentially the same phenotype as *plt1-1* (Figure 1G; see below). We therefore concluded that At3g20840 is the *PLT1* gene.

In situ RNA hybridization detected a *PLT1*-specific signal in the QC, surrounding stem cells, and the uppermost layer of differentiated columella cells, similar to the GUS staining pattern of the *plt1-1* promoter trap line (compare Figures 1D and 1E). No signal was detected in *plt1-1* homozygotes (Figure 1F), indicating that this allele causes a severe reduction of expression of the gene and may be a null. In *plt1-2* homozygotes, the signal was highly reduced but still detectable, consistent with the weaker phenotype of this allele (data not shown).

We isolated a full-length *PLT1* cDNA (GenBank AY506549). The predicted PLT1 protein is a member of the AINTEGUMENTA-like (AIL) subclass of the AP2/EREBP family of transcription factors (Riechmann and Meyerowitz, 1998; B. Krizek, personal communication)

and contains two repeats of the conserved AP2 DNA binding domain (residues 179–255 and 281–349) and the conserved linker region (residues 256–280) (Figure 1G and Supplemental Figure S1 at http://www.cell.com/cgi/content/full/119/1/109/DC1/). A bipartite nuclear targeting sequence is predicted in residues 364–380. A GFP-PLT1 fusion protein preferentially localizes to the nucleus in the RM, consistent with the proposed function of PLT1 as a transcription factor (Figure 1H).

The closest homolog of *PLT1* (At1g51190) resides in a region of segmental genome duplication (http://wolfe.gen.tcd.ie/athal/dup), and we named this gene *PLT2* (Figure 1G). The protein sequence deduced from a *PLT2* cDNA (GenBank AY506550) is strikingly similar to PLT1 with 97.7% amino acid identity in the region spanning both AP2 domains (Supplemental Figure S1 on the *Cell* website). *PLT2* displays essentially the same expression pattern as *PLT1* (Figure 1I) and a GFP-PLT2 fusion localizes to the nucleus (data not shown). In RT-PCR analysis, *PLT1* and *PLT2* transcripts were mainly detected in roots, but very weakly in flowers and not in leaves, indicating that *PLT* expression is strongly associated with root identity (data not shown).

Mutations in Single *PLT* Genes Cause Subtle Defects in Distal Cell Division Patterns and Root Cell Proliferation

To examine the postembryonic function of the *PLT* genes, independent insertion alleles were analyzed. *plt1* mutations affect cell division patterns in the QC and columella root cap. In wild-type at 2 days postgermination (dpg), the columella has four tiers of cells, consisting of one stem cell tier (tier1) and three tiers of differentiated cells marked by accumulation of starch granules (tier2–4; Figure 2A; Table 1). In some plants, a fraction of the stem cells may have divided to produce daughters that will accumulate starch granules. By contrast, *plt1* mutants displayed an increased number of columella tiers, mainly due to enhanced cell division in tier1 (Figure 2B; Table 1). In addition, we frequently found extra cells in tier2 of the columella (24 of 48 plants in *plt1-4*; arrowhead in Figure 2B), which never occurs in wild-type ($n = 247$). These extra cells could be derived either from ectopic cell divisions in tier2 or from irregular and unsynchronized cell divisions in tier1. In addition to the columella defect, we occasionally observed extra QC cells in *plt1* mutants (Table 1).

In *plt2-1* mutants, we could not detect significant abnormalities in columella or QC organization. However, the *plt2-2* mutant contains increased columella cell numbers, although the frequency of extra tiers is lower compared to that of *plt1* (Figure 2C; Table 1) and no extra cells in tier2 are present ($n = 339$). The subtle defects in the columella region of *plt2-2* mutants resemble those observed in *plt1*.

To assess whether mutations in *PLT* genes affect cell division in the RM, we measured the root length and the number of meristematic cells. Both *plt1* and *plt2* single mutants display a slight but significant reduction in the growth rate of the root and in the number of meristematic cells (Figure 2E; Supplemental Figures S2A and S2B on the *Cell* website). The average length of differentiated cells in the root is not significantly altered or even slightly increased (Supplemental Figure S2C online), suggesting that the reduction in root growth is due to reduced production of cells in the RM.

PLT1 and *PLT2* Are Redundantly Required for Distal Cell Division Patterns and Stem Cell Maintenance in the Root Meristem

The high homology between the two *PLT* genes and their similar expression patterns suggest redundant roles in root development. We therefore constructed double mutants of *plt1* and *plt2*, using four different allelic combinations (*plt1-3 plt2-2*, *plt1-4 plt2-1*, *plt1-4 plt2-2*, and *plt1-5 plt2-1*). All double mutants display essentially the same defects. The columella contains more cells, its stratified structure is disturbed, and starch granules accumulate in all columella layers including cells at the position of the stem cells (Figure 2D). Root growth is extremely reduced compared to wild-type and each single mutant (Figure 2E; Supplemental Figure S2A online), and the number of meristematic cells is also highly reduced (Figures 2F and 2G; Supplemental Figure S2B on the *Cell* website). Consistently, expression of cyclin-GUS, a marker for the G2/M phase of the cell cycle (Colón-Carmona et al., 1999), is detected in a smaller area along the longitudinal axis of *plt1 plt2* (Figures 2H and 2I; 144.0 μm $\pm$ 4.1 SEM in wild-type Col, $n = 56$; 35.1 μm $\pm$ 2.6 SEM in *plt1-4 plt2-2*, $n = 73$; 3 dpg), indicating that the population of dividing cells is smaller. Furthermore, the size of the RM in double mutants rapidly decreases and eventually all cells in the RM differentiate at 6 to 8 dpg, as evident by formation of root hair cells and xylem strands at the root tip (Figures 2J and 2K). These results show that *PLT1* and *PLT2* are redundantly required for distal cell division patterns and RM maintenance.

The reduced root growth of the double mutant could be due to a reduction either in the rate of cell production or in the final size of mature cells. Interestingly, the size of mature cortex cells in *plt1 plt2* is reduced to nearly a half of that of wild-type (Supplemental Figure S2C online). This finding, however, only partially accounts for the reduction of whole root length (approximately 1/8 of wild-type at 8 dpg; Supplemental Figure S2A), showing that the production of cells in the RM is significantly decreased (to $\sim$1/4 of wild-type) in the double mutant.

The *plt1 plt2* mutant produces numerous lateral roots from the pericycle of the primary root. The RM of these lateral roots is highly reduced in size, contains disorganized columella layers, and differentiates shortly after initiation (Figures 2L and 2M), similar to the primary RM. Terminated primary and secondary roots of *plt1 plt2* continue to produce lateral roots of higher orders, resulting in a more branched root system compared to that of wild-type (Figures 2N and 2O). These observations indicate that the *PLT* genes are also required for RM maintenance in lateral roots, but not for lateral root initiation. We observed no obvious phenotype in *plt1 plt2* shoots (Figures 2N and 2O).

To assess whether the loss of stem cells is associated with mis-specification of the QC in the double mutant, we examined three independent QC markers in *plt1-4 plt2-2* at a stage where the RM is still present in the primary root (3 dpg). Expression of QC25 is either com-

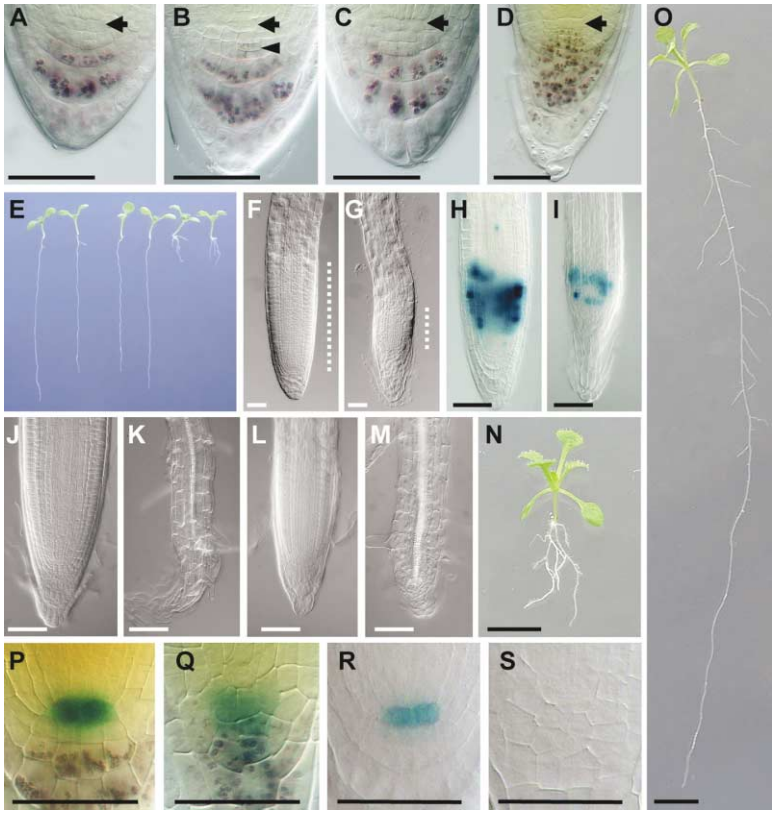

Figure 2. The *PLT* Genes Are Required for QC Identity and Stem Cell Maintenance

(A–D) Distal RM region at 2 dpg of wild-type WS (A), *plt1-4* (B), *plt2-2* (C), and *plt1-4 plt2-2* (D). Starch granule staining marks differentiated columella cells (brown). Arrows mark the position of QC, arrowhead in (B) marks extra cell in uppermost layer of differentiated columella.

(E) 8 dpg seedlings. From left to right: WS, *plt1-3*, *plt1-4*, *plt2-2*, *plt1-3 plt2-2*, *plt1-4 plt2-2*.

(F and G) Two dpg root tip of WS (F) and *plt1-4 plt2-2* (G). Dotted bars indicate the size of meristematic region.

(H and I) Cyclin-GUS expression in wild-type Col (H) and *plt1-4 plt2-2* (I) at 3 dpg.

(J and K) Eight dpg root tip of WS (J) and *plt1-4 plt2-2* (K).

(L and M) Tip region of lateral root in WS (L) and *plt1-4 plt2-2* (M) at 14 dpg.

(N and O) 12 dpg *plt1-4 plt2-2* (N) and WS (O) plant.

(P and Q) Double staining of the QC25 GUS marker (blue) and starch granules (dark brown) in WS (P) and *plt1-4 plt2-2* (Q) at 3 dpg.

(R and S) GUS staining of QC184 in WS (R) and *plt1-4 plt2-2* (S) at 3 dpg.

Scale bars for (A) to (D), (F) to (M), and (P) to (S), 40 μm. Scale bars for (N) and (O), 5 mm.

pletely missing (37.2%, n = 94) or reduced. When QC25 is expressed, the signal is expanded to adjacent cells with starch granules, again suggesting that wild-type columella stem cells are not present (Figures 2P and 2Q). Most plants fail to express QC46 (86.5%, n = 37) or express this marker only weakly. The third marker, QC184, is either completely missing (61.4%; n = 83; Figures 2R and 2S) or highly reduced. These three markers are expressed in the QC of all wild-type WS plants (n > 100). These results indicate that *plt* double mutants fail to establish or maintain QC cells with correct identity and in this way interfere with the maintenance of RM stem cells.

PLT Genes Are Expressed in the Early Basal Embryo Domain and Required for Embryonic Specification of the Stem Cell Niche

To investigate whether the *PLT* genes are required for initiation of the stem cell niche in the embryo, we examined their embryonic expression and function. *PLT1* mRNA is detectable as early as the octant stage in the basal half of the embryo proper (Figure 3A). At the globular stage, *PLT1* is expressed in the provascular cells and in the lens-shaped QC progenitor cell derived from the hypophysis (Figure 3B). As embryogenesis proceeds, *PLT1* expression becomes restricted to the distal part of the embryonic root, including the QC and surrounding stem cells (Figure 3C). *PLT2* displays a very similar expression pattern at all stages examined except that its expression domain expands laterally to include some ground tissue cells (Figure 3D).

In the mature embryo stage, the *plt1-4* and *plt2-2* single mutants do not reveal any phenotype (data not shown) while the double mutant displays abnormal cellular organization of the hypophyseal derivatives. In wild-type, this region consists of the QC, columella stem cells, and two basal cell tiers which will become differentiated columella layers (Figure 3E). Some of the columella stem cells may have divided to produce daughters (Figure 3E, arrowheads). The corresponding region of

Table 1. Columella and QC Defects in *plt* Single Mutants

	WS	*plt1-1*	*plt1-3*	*plt1-4*	*plt2-2*
Average Number of Columella Tiers[a] (±SEM)	4.25 (±0.03)	5.00 (±0.00)	5.06 (±0.02)	5.05 (±0.02)	4.49 (±0.03)
n =	247	15	204	348	339
Number of Extra Cells in the QC (±SEM)	0.027 (±0.032)	0.467 (±0.165)	0.464 (±0.120)	0.417 (±0.083)	0.149 (±0.074)
n =	37	15	28	48	47

[a] Includes tier1.

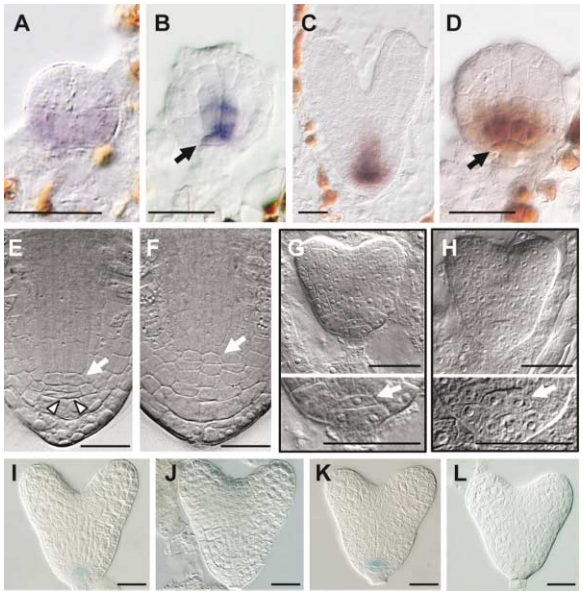

Figure 3. The *PLT* Genes Specify the Stem Cell Niche during Embryogenesis

(A–D) In situ hybridization of *PTL1* at the 8 cell (A), globular (B), and torpedo (C) stages, and *PLT2* at the globular stage in wild-type (D). Arrows in (B) and (D) indicate the lens-shaped QC progenitor cell.

(E and F) Cellular organization in wild-type WS (E) and *plt1-4 plt2-2* (F) mature embryo, with arrows indicating cells corresponding to the QC.

(G and H) Cellular organization in WS (G) and *plt1-4 plt2-2* (H) embryo at the heart stage. Lower panels show magnification of basal regions with arrows indicating QC progenitor cells.

(I–L) QC25 expression in heart stage embryo of WS (I) but absent in *plt1-4 plt2-2* (J); QC46 expression in WS (K) but absent in *plt1-4 plt2-2* (L).

Scale bars, 20 μm.

plt1 plt2 contains more cells with a less stratified organization (Figure 3F). At the early heart stage, the wild-type hypophysis has formed an upper lens-shaped cell and lower cells that have divided vertically (Figure 3G). The lens-shaped cell will undergo vertical divisions and form the QC while the lower cells will divide horizontally in the next round of cell division (Scheres et al., 1994). By contrast, the lens-shaped cell in *plt1 plt2* heart stage embryos is enlarged and has already performed vertical cell divisions (Figure 3H). Furthermore, the lower cells divide vertically or obliquely rather than horizontally.

To assess cell identity in the embryonic stem cell region, we examined QC marker expression. In wild-type, expression of QC25 starts at the mid-heart stage, whereas QC46 expression starts at the early heart stage in the lens-shaped cell and its derivatives (Figures 3I and 3K). QC184 is initially detected at the early torpedo stage in the derivatives of the lens-shaped cell (data not shown). In *plt1 plt2*, neither QC25 nor QC46 expression is detected throughout embryogenesis (Figures 3J and 3L). QC184 is detected only at the mature stage but not in earlier stage embryos (data not shown). Our results show that *PLT* function is required for early stages of QC specification and for correct cell division patterns of neighboring cells; these results correlate well with the restriction of *PLT* expression to the stem cell niche around the heart stage of embryogenesis.

PLT1 and PLT2 Act in Parallel with the SHORTROOT/SCARECROW Pathway to Pattern the Stem Cell Niche

The GRAS family transcription factors SCR and SHR are also required for QC specification and stem cell maintenance in the root and both are essential for QC25 and QC46 expression (Sabatini et al., 2003), suggesting that target genes of this pathway overlap with those of PLT. We therefore investigated whether the SHR/SCR and PLT pathways influence the transcription or function of each other.

SCR expression in the *plt1-4 plt2-2* background was examined using a YFP reporter driven by the *SCR* promoter (*pSCR-YFP*). In wild-type, *pSCR-YFP* is expressed in the QC and endodermis cells of the postembryonic RM (Figure 4A) as well as in the embryo (Figure 4C). In *plt1 plt2*, *SCR* expression is detected in the corresponding cells both in embryonic and in early postembryonic roots (Figures 4B and 4D), indicating that the *PLT* genes are not required for *SCR* expression. In wild-type, columella stem cells adjacent to the QC are maintained by *SCR* activity in the QC and do not accumulate starch granules (Sabatini et al., 2003; arrowhead in Figure 4A). In *plt1 plt2*, however, cells adjacent to *SCR*-positive cells accumulate starch granules (arrowhead in Figure 4B), confirming that these cells do not possess correct columella stem cell identity.

SHR expression was examined using a SHR-GFP fusion protein driven by the *SHR* promoter (Nakajima et al., 2001). In wild-type QC and endodermal cells, SHR-GFP is present in the nucleus (Figures 4E and 4G). In *plt1 plt2*, we found nuclear accumulation of SHR-GFP in the equivalent cells both in postembryonic and embryonic roots (Figures 4F and 4H), showing that the *PLT* genes are not essential for the expression of the SHR protein. SHR-GFP and *SCR* promoter activity is sometimes lacking in cells that correspond to the QC in the postembryonic root (arrowhead in Figure 4F; data not shown), which may reflect a secondary effect of the cessation of meristem activity or a requirement for PLT activity in the maintenance of SHR and SCR expression.

To examine the effect of the *SCR/SHR* pathway on *PLT* mRNA accumulation, we next analyzed *PLT1* expression in the *scr-1* and *shr-1* mutants by in situ hybridization. *PLT1* transcript is still present in both of these strong alleles, although the signal intensity is lower compared to the wild-type control (Figures 4I to 4K). These results show that neither *SCR* nor *SHR* are essential for *PLT1* transcription.

Although the *SCR/SHR* and *PLT* expression domains are established independently, the proteins could post-transcriptionally modify each other's activity. To test whether the SCR/SHR pathway solely acted on stem cell maintenance by modulating PLT activity or vice versa, we constructed *scr-4 plt1-4 plt2-2* and *shr-1 plt1-4 plt2-2* triple mutants. Similar to the *plt1 plt2* double mutant, *scr* and *shr* single mutants display reduced RM size and differentiation of RM cells (Sabatini et al., 2003). In both triple mutant combinations, cells in the RM differentiate earlier compared to any of the *scr* and *shr* single or *plt1 plt2* double mutants (Figures 4L–4P). These results indicate that the *PLT* genes are still active in the *scr* and *shr* backgrounds and, conversely, that *SCR* and *SHR* are active in the *plt1 plt2* double mutant

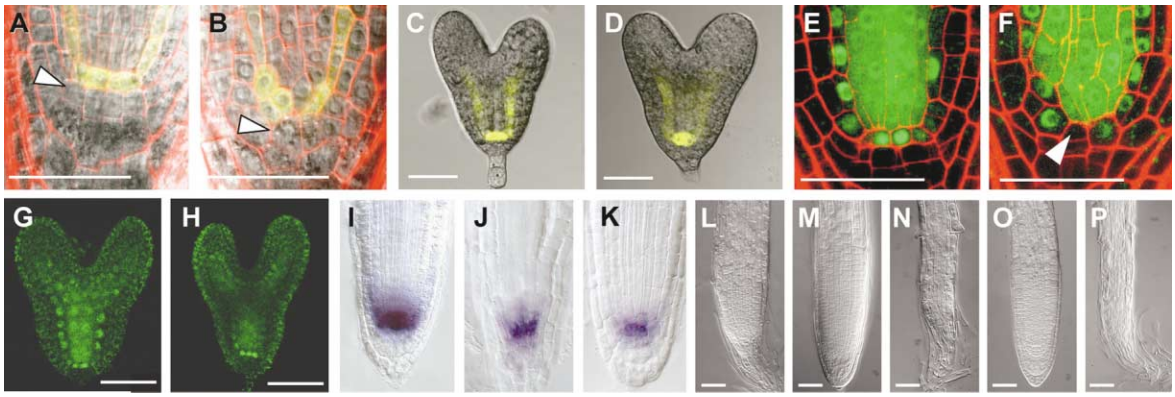

Figure 4. The *PLT* Genes Act Independently of *SCR* and *SHR* in Stem Cell Maintenance

(A and B) *pSCR-YFP* expression in WS (A) and *plt1-4 plt2-2* (B) at 3 dpg. Cells immediately below YFP-expressing cells (arrowheads) accumulate starch granules (dark particles) in *plt1 plt2* but not in wild-type.

(C and D) *pSCR-YFP* expression in heart stage embryo of WS (C) and *plt1-4 plt2-2* (D).

(E and F) GFP-SHR accumulation in 3 dpg seedlings of control (E) and *plt1-4 plt2-2* (F). Some cells at the QC position lack GFP-SHR (arrowhead).

(G and H) GFP-SHR accumulation in heart stage embryo of control (G) and *plt1-4 plt2-2* (H).

(I–K) Whole-mount in situ hybridization of *PLT1* in WS (I), *scr-1* (J), and *shr-1* (K) root at 3 dpg.

(L–P) 3 dpg root of *plt1-4 plt2-2* (L), *scr-4* (M), *scr-4 plt1-4 plt2-2* (N), *shr-1* (O), and *shr-1 plt1-4 plt2-2* (P).

Scale bars, 40 μm.

to maintain stem cells in the root. Taken together, our data argue that the *PLT* and *SCR/SHR* pathways provide parallel inputs in root stem cell niche specification. The two pathways converge at a subset of target promoters (as indicated by the same effect on QC25 and QC46) but remain independent at others (as revealed by QC184, which is dependent on *PLT* but not on *SCR/SHR*).

PLT Expression Responds to Changes in Auxin Level or Distribution and Depends on Auxin Response Factors

The accumulation of auxin at the distal tip of root primordia is associated with QC and stem cell specification. We tested whether the *PLT* genes are required for the generation or perception of this auxin maximum. The auxin responsive reporter *DR5-GUS* visualizes the auxin response maximum in the wild-type RM, where strong GUS activity is detected in the QC, columella stem cells, and differentiated columella and weak expression in vascular cells adjacent to the QC (Figure 5A; Sabatini et al., 1999). *plt1-4 plt2-2* mutants reveal essentially the same pattern but lack the weak staining in vascular cells (Figure 5B). Overnight treatment of roots with the synthetic auxin 2,4-D induces *DR5-GUS* in all RM cells of both wild-type and *plt1-4 plt2-2* (Figures 5C and 5D). Since neither auxin distribution nor primary auxin response is significantly impaired in the double mutant, we concluded that the *PLT* genes do not strongly affect these processes.

To examine whether the *PLT* genes act downstream of auxin accumulation, we first monitored *PLT* mRNA levels upon addition of auxins using semiquantitative RT-PCR (Figure 5E). In contrast to mRNA of the early auxin response gene *IAA2*, *PLT1* and *PLT2* transcripts are not elevated 5 hr after auxin application, suggesting that the *PLT* genes are not primary auxin response genes. However, both transcripts increase between 5 and 24 hr after application of 50 μM IAA or NAA. Our results indicate that auxin accumulation promotes *PLT* transcription in domains with prolonged auxin response.

The spatial correlation of *PLT* transcription with high levels of *DR5-GUS* reporter activation suggested that *PLT* expression could be dependent on transcription factors of the ARF class that bind to the auxin-responsive elements in this reporter. *MONOPTEROS* (*MP*), which encodes a member of this class, is expressed throughout the early embryo proper. *MP* and *PLT* expression expand together into the lens-shaped QC progenitor cell at the globular stage when *PLT* transcripts first accumulate there (Hamann et al., 2002). In embryos of the strong *mp-T370* allele at 8- to 16-cell stages, both *PLT* genes are expressed (Figure 5F; data not shown). At late globular to early heart stages, expression of *PLT* is lost in the majority of *mp* embryos (79%, n = 24 for *PLT1* and 83%, n = 23 for *PLT2*; Figures 5G–5I), whereas all wild-type siblings examined express *PLT* (n = 37 for *PLT1* and n = 25 for *PLT2*). The partial independence of embryonic *PLT* expression on *MP* might be caused by the redundantly acting *MP* homolog *NON-PHOTOTROPIC HYPOCOTYL4* (*NPH4*) (Hardtke et al., 2004). Consistent with this notion, heart stage double mutant embryos display further reduction of *PLT1* transcript (90% completely lost, 10% extremely reduced, n = 32) whereas *nph4* single mutant embryos contain normal transcript levels (Figures 5J and 5K).

To circumvent redundancy in ARF action during embryogenesis, we investigated a role of the auxin response transcriptional machinery in the initiation of *PLT1* transcription in lateral roots. To this end, we used the *plt1-1* promoter trap whose activity initiates in progenitor cells of lateral root primordia in the pericycle of wild-type (Figures 5L and 5M) to examine promoter trap activity in plants carrying the dominant *solitary-root-1* (*slr-1*) mutation. These plants accumulate a mutant IAA14 protein as a repressor of ARF-mediated transcrip-

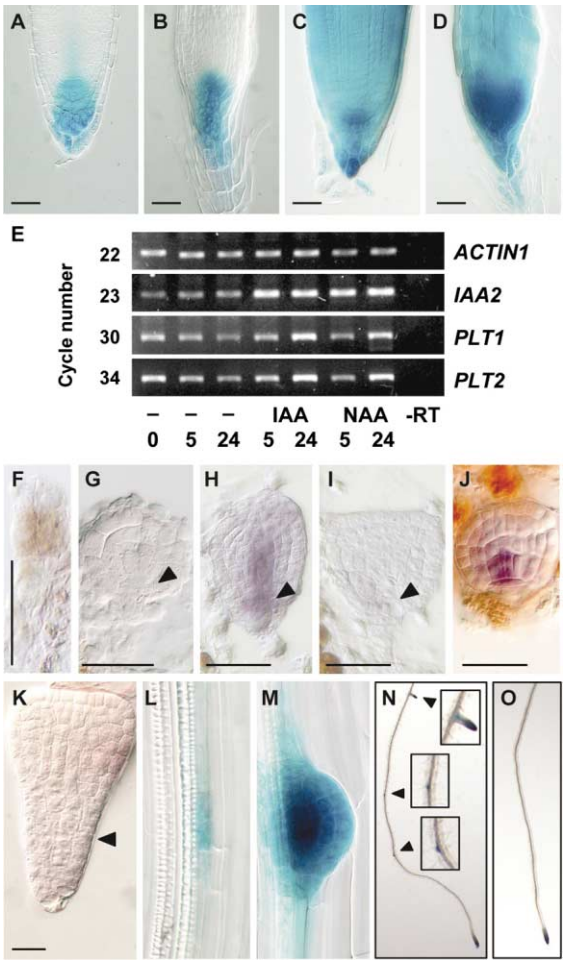

Figure 5. *PLT* Expression Pattern Depends on Auxin and Auxin Response Factors

(A and B) *DR5-GUS* expression in wild-type Col (A) and *plt1-4 plt2-2* (B) at 3 dpg.

(C and D) Response of *DR5-GUS* to overnight treatment with 5 μM 2,4-D in Col (C) and *plt1-4 plt2-2* (D) at 3 dpg.

(E) Semiquantitative RT-PCR of *ACTIN1* (control), *IAA2*, *PLT1*, and *PLT2*. RNA was isolated from seedlings with 5 or 24 hr after mock (—), 50 μM IAA, or 50 μM NAA treatments. No PCR product was detected from samples without reverse transcription (-RT).

(F) In situ hybridization of *PLT1* in *mp-T370* preglobular stage embryo.

(G–I) In situ hybridization of *PLT1* (G and H) and *PLT2* (I) in *mp-T370* embryos at the globular to transition stages, showing the absence (G and I) or presence (H) of signals. Arrowheads indicate hypophyseal derivatives giving rise to basal peg structure.

(J and K) In situ hybridization of *PLT1* in *nph4-1* (J) and *mp-G12 nph4-1* (K) embryo at the transition stage. Arrowhead in (K) indicates a large basal peg structure.

(L and M) GUS staining of *plt1-1* promoter trap in lateral root precursor cells (L) and emerging primordium (M).

(N and O) GUS staining of *plt1-1* promoter trap line introduced into the Col (N) and *slr-1* (O) backgrounds. *PTL1* expression is detected in the pericycle in Col (insets in N) but not in *slr-1*.

Scale bars, 30 μm.

tional responses to auxin, resulting in an early and specific block in lateral root formation (Fukaki et al., 2002). We detected promoter trap activity in early lateral root primordia emerging from pericycle cells of plants wild-type for *SLR* (3.77 per root, n = 26; arrowheads in Figure 5N). No activity was detected in the pericycle of *slr-1*/+ plants (Figure 5O), except in rare "escape" lateral root primordia (0.18 per root, n = 28). Agilent gene chip data on the auxin inducibility during lateral root development showed that the *PLT* genes were 7- to 9-fold inducible by auxin within 24 hr in wild-type whereas this induction was severely impaired in the *slr-1* mutant background (H. Fukaki, personal communication). As *PLT1* expression appears to precede the early *SLR*-dependent morphological changes that occur in lateral root primordia, these data suggest that activation of *PLT1* transcription in lateral roots occurs downstream of auxin response factors.

To assess whether auxin accumulation can promote stem cell specification through a pathway operating independently of the *PLT* genes, we cultured *plt1 plt2* seedlings carrying the QC25 promoter trap in 0.05, 0.5, 5, and 50 μM IAA or NAA. After 24 and 72 hr, we observed no rescue of stem cell activity in *plt1 plt2* and the residual QC25 expression was never restored to wild-type levels ($n > 10$ for each treatment), indicating that auxins cannot bypass the requirement for the *PLT* genes.

Ectopic Embryonic *PLT* Gene Activity Produces Ectopic Stem Cell Niches and Homeotic Transformations to Root and Hypocotyl Identity

We investigated whether the *PLT* genes could promote stem cell niche formation in ectopic positions. To this end, we induced ubiquitous expression of the *PLT* genes from early embryogenesis onward using the promoter for the *RIBOSOMAL PROTEIN S5A* (*pRPS5A*; Weijers et al., 2001) in combination with the GAL4/UAS-based two-component expression system (Brand and Perrimon, 1993). The *RPS5A* promoter is active at all stages and in all cells of the embryo proper (Weijers et al., 2001). We generated activator lines in which a modified GAL4 protein is expressed under the control of the *RPS5A* promoter (*pRPS5A-GAL4*) and target lines carrying *PLT1* or *PLT2* coding sequences downstream of the *UAS* promoter (*UAS-PLT*). To visualize GAL4 activity, we engineered the *YFP* coding sequence downstream of a *UAS* copy into the target constructs. These parental lines revealed no developmental abnormalities.

Twelve *UAS-PLT2* lines were generated and crossed with *pRPS5A-GAL4* driver lines. Two of the *UAS-PLT2* lines did not cause any specific phenotype. Three lines produced seedlings with root stem cell termination, which was likely caused by cosuppression of *PLT* genes (data not shown). Seven other lines yielded novel phenotypes, ubiquitous YFP fluorescence, and increased *PLT2* expression as judged by semiquantitative RT-PCR (Supplemental Figure S3 on the *Cell* website). Among them, six were categorized as phenotypically strong and one as mild. The apical region of the strong lines lacks a SAM (data not shown) and branches into continuously proliferating clusters of green structures (Figure 6A), while the corresponding region of wild-type gives rise to the SAM and two cotyledons. These green structures resemble cotyledons or leaves but are smaller and often have radial rather than bilateral symmetry (data not shown). The base of each branch, at the position of the cotyledon petiole in wild-type, consists of white root-

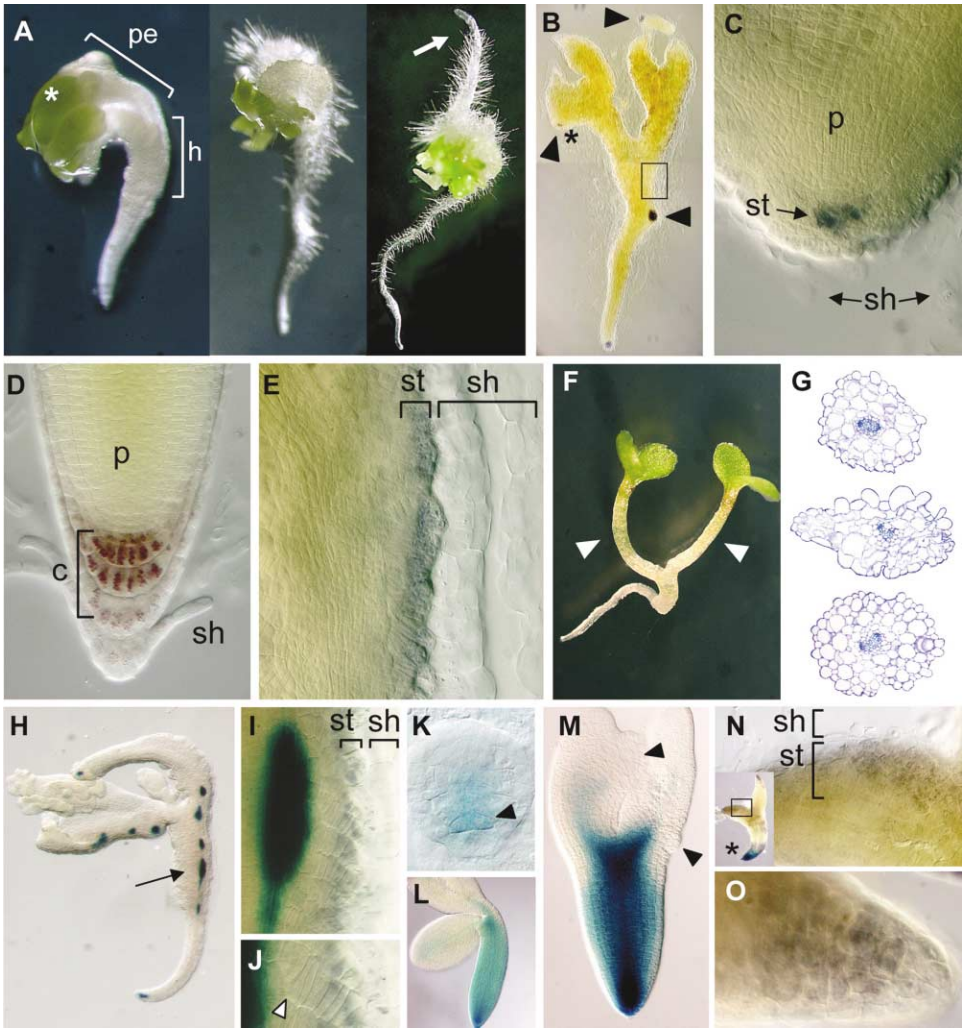

Figure 6. *PLT* genes Are Involved in Embryonic Root and Hypocotyl Specification

(A) Ectopic root phenotype induced by cross between *pRPS5A-GAL4* and strong *UAS-PLT2* lines. Three subsequent developmental stages of a single seedling. Asterisk indicates clusters of green structures. (pe) and (h) indicate regions corresponding to petiole and hypocotyl, respectively. Arrow indicates ectopic root emerging from the apical region.

(B) Seedling covered with distal root cell types induced by cross between *pRPS5A-GAL4* and strong *UAS-PLT2* lines. Arrowheads indicate clusters of starch granule-accumulating cells.

(C) Magnification of the region indicated with asterisk in (B). Elongated provascular-like cells (p) and shedding root cap-like cells (sh) surround the starch-accumulating cells (st).

(D) The distal region of a wild-type root with starch-accumulating columella cells (c) flanked by provascular (p) and shedding root cap cells (sh).

(E) Magnification of the region indicated with rectangle in (B), displaying starch-accumulating cells (st) and shedding cells (sh).

(F) Seedling with ectopic hypocotyl at cotyledon petiole region (arrowheads) induced by cross between *pRPS5A-GAL4* and mild *UAS-PLT2* lines.

(G) Transverse sections of wild-type hypocotyl (top), wild-type petiole (middle), and ectopic hypocotyl in *pRPS5A-GAL4/UAS-PLT2* (bottom).

(H) QC25 expression in *pRPS5A-GAL4/UAS-PLT1* seedling with ectopic distal-root cell phenotype.

(I) Double staining of GUS and starch granules in the region indicated with arrow in (H). st, starch-accumulating cells; sh, shedding cells.

(J) Magnification of (I) showing dividing cells that display neither GUS nor starch staining (arrowhead).

(K and L) Auxin-sensitive IAA2-GUS expression in wild-type embryos at the globular (K) and mature (L) stages. Arrowhead in (K) indicates the QC progenitor cells.

(M) IAA2-GUS expression in *pRPS5A-GAL4/UAS-PLT2* embryo. Embryo was overstained to ensure the absence of GUS activity in the ectopic outgrowths (arrowheads).

(N and O) Double staining of IAA2-GUS activity and starch granules in root-like structures on explanted *pRPS5A-GAL4/UAS-PLT2* embryos. Inset in (N) shows an entire explanted embryo with GUS staining in the primary root (asterisk) but not in the magnified area (rectangle). st, starch-accumulating cells; sh, shedding cells.

like tissue (Figure 6A, left panel). Numerous root hairs emerge from the surface of this region as well as from the hypocotyl (Figure 6A, middle panel). In addition, ectopic roots with fully active RMs frequently emerge from the flank of the apical part (arrow in Figure 6A, right panel). These results indicate that ectopic expression of *PLT2* prevents normal hypocotyl, cotyledon, and SAM formation and induces ectopic roots with active stem cells.

The strong *UAS-PLT2* target lines occasionally yield seedlings that entirely consist of white tissues with severely retarded growth. In these seedlings, clusters of starch granule-accumulating cells, which resemble wild-type columella root cap, are produced ectopically in apical regions (Figure 6B, arrowheads). These clusters are often associated with the apical end of branches or bulges forming on the lateral surface of the seedling. Interestingly, the clusters are flanked distally by shedding cells and proximally by cells elongating along the axis of the bulge, resembling shedding root cap cells and provascular cells in the wild-type root tip, respectively (Figures 6C and 6D). Moreover, virtually the whole surface of these seedlings is covered with shedding cells and subtending layers of starch granule-accumulating cells (Figure 6E). These observations indicate that constitutive expression of *PLT2* can cause ectopic formation of distal RM cells in an arrangement comparable to wild-type.

In progeny from crosses with the mild *UAS-PLT2* line, the apical part of seedlings bifurcates to form hypocotyl-like structures with a distal SAM between a pair of cotyledons (Figure 6F). The hypocotyl-like structures display a radial anatomy with vascular organization and symmetric ground tissue layers similar to wild-type hypocotyl (Figure 6G), indicating a transformation from petiole to hypocotyl identity.

When eleven target lines harboring *UAS-PLT1* were generated and crossed to *pRPS5A-GAL4* activator lines, one line displayed a range of phenotypes encompassing production of clusters of green leaf-like organs on the surface of cotyledons, ectopic root-like structures in place of cotyledons, and ectopic columella cells covering the seedling surface, similar to strong *UAS-PLT2* lines. However, none of the *UAS-PLT1* lines displayed ectopic hypocotyl identity. To investigate whether *PLT1* can also induce ectopic hypocotyl identity, we directly transformed a *pRPS5A-GAL4* activator line with *UAS-PLT1* target construct and examined T1 seedlings. Among 38 transformants, 3 displayed ectopic hypocotyl identity, 12 displayed ectopic root identity, and 3 were covered with ectopic columella cells. All these phenotypes are associated with ubiquitous YFP fluorescence.

We next asked whether ectopic *PLT*-induced stem cells are associated with ectopic QC specification by using the QC25 promoter trap. In wild-type seedlings, QC25 is exclusively expressed in the QC (Figure 2P) but not in any other region (data not shown). When plants harboring both *UAS-PLT1* and QC25 are crossed with *pRPS5A* activator lines, the resulting F1 seedlings display regions of strong QC25 expression (Figure 6H). Cells that ectopically express QC25 are always located internally to cells that accumulate starch granules (Figure 6I). Between these two cell types, we often found cells that express neither the QC25 nor starch granule markers (Figure 6J, arrowhead). The orientation of cell walls suggests that these cells proliferate along the axis between QC25- and starch granule-expressing cells, similar to wild-type columella stem cells.

Taken together, our results show that *PLT1* and *PLT2* can ectopically induce all organ identities that originate from the basal region of the embryo, i.e., hypocotyl, root, and root stem cell niche. To investigate whether the ectopic activation of root stem cell activity by *PLT* genes was accompanied by new sites of auxin accumulation, we combined the relevant *pRPS5A-GAL4* and *UAS-PLT2* lines with the sensitive auxin-responsive reporter line *IAA2-GUS* (Swarup et al., 2001). IAA2-GUS marks the incipient root primordium from globular stage onward in wild-type embryos (Figures 6K and 6L) and in those ectopically expressing *PLT2* (Figure 6M). In the latter, newly formed ectopic primordia stain neither in freshly harvested embryos (arrowheads in Figure 6M) nor in explanted embryos which develop regions with shedding root cap-like cells (Figure 6N) and ectopic primordia with characteristic root anatomy (Figure 6O). These results suggest that the embryonic induction of ectopic root stem cell groups by *PLT* genes does not require auxin accumulation.

Discussion

A Patterning Model for the Root Stem Cell Niche

We have identified two members of the AP2 domain transcription factor family, PLT1 and PLT2, which are redundantly required for the embryonic specification of stem cell organizing QC cells and for the maintenance of root stem cells. Accordingly, *PLT* transcription is detected in the stem cell niche. *PLT* mRNA distribution associates with an auxin maximum previously implicated in the specification of distal cell types in the root including QC and stem cells (Sabatini et al., 1999; Friml et al., 2002) and embryonic *PLT* transcription requires the ARFs MP and NPH4 at the stage when the stem cell niche is specified. Ectopic expression of *PLT* genes in the embryo results in the ectopic accumulation of QC and associated root stem cells. Ectopic QC cells in *PLT* overexpression lines appear to originate from cells adjacent to the stele (Di Laurenzio et al., 1996; Nakajima et al., 2001) in line with the requirement for SHR and SCR to specify the QC (Sabatini et al., 2003), suggesting that PLT and SCR/SHR accumulation is necessary and sufficient for specification of the stem cell niche. Our data suggest that *SHR*/*SCR* and *PLT* genes provide parallel input into QC and stem cell specification, which leads to a model that explains how the small group of cells of the *Arabidopsis* root stem cell niche is specified in a precise position by a combination of distal and radial cues (Figure 7). First, ARFs maintain *PLT* expression in the basal region of the developing globular embryo. Second, the SHR transcription factor accumulates in provascular cells through an as yet unidentified mechanism, moves to a single adjacent cell layer, and promotes transcription of *SCR*. Third, the PLT, SHR, and SCR proteins jointly specify the QC. In this process, SCR is cell-autonomously required for QC specification (Sabatini et al., 2003) and determines precisely which cell layer within the PLT expression domain acquires QC identity. Fourth, cells surrounding the QC also express PLT and become specified as stem cells by means of yet unidentified short-range signals from the QC (van den Berg et al., 1997).

The *PLETHORA* Genes as Developmental Effectors of Auxin Accumulation Patterns?

Differential auxin accumulation has been correlated to a number of developmental responses during organ out-

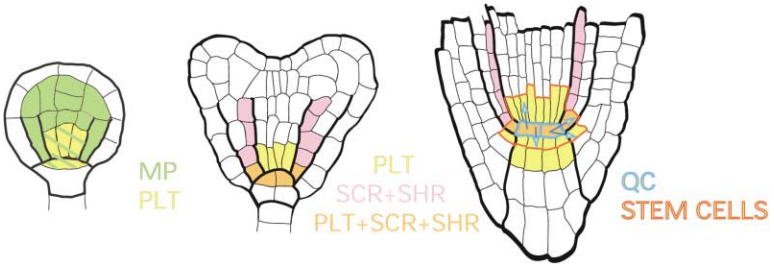

Figure 7. A Model for Patterning and Maintenance of the Root Stem Cell Niche

Early expression of *PLT* is maintained by the MP and NPH4 auxin response factors within their basal expression domain (left). Cells that express PLT, SCR, and SHR are specified to become the QC (middle). The QC signals to the surrounding cells to maintain their stem cell activity (right).

growth (Sabatini et al., 1999; Benková et al., 2003; Friml et al., 2003b; Reinhardt et al., 2003). Auxin activates transcription of a diverse set of target genes, consistent with the notion that long-term effects of auxin, which involve cell fate or patterning processes in many cases, rely on transcriptional regulation.

Here, we provide evidence that auxin response and *PLT* transcription are spatially correlated in the wild-type embryo, primary root, and emerging lateral root primordium (Sabatini et al., 1999; Benková et al., 2003; Friml et al., 2003b). In addition, *PLT* transcript accumulation is elevated by application of auxin and is dependent on ARF transcription factors. These findings are consistent with the idea that auxin distribution is the spatial input for *PLT* gene expression. Moreover, the *pin2 pin3 pin4 pin7* quadruple mutant defective in the redundantly acting PIN polar auxin efflux facilitators shows abundant *PLT* transcript accumulation throughout the embryo proper from pre-globular stage onward (I. Blilou et al., submitted). This observation strongly supports that PIN-mediated basal restriction of auxin accumulation regulates *PLT* transcript distribution in the embryo. Technical issues complicate a rigorous test whether early embryonic *PLT* transcript accumulation is dependent on redundant ARF transcription factors. However, the SLR-dependent *PLT1* promoter trap activation in the earliest stages of lateral root primordium as well as the SLR-dependent induction of *PLT* transcript accumulation by auxins observed on microarrays suggest that the induction of *PLT* transcription in lateral root primordia requires the ARF/AUX-IAA machinery. It is noteworthy, however, that the response of *PLT* transcription to auxin occurs significantly later than that of primary auxin response genes.

Our expression analysis provides correlative evidence that auxin accumulation associates with *PLT* gene regulation in root formation. Three further observations suggest that this link is causal: (1) loss of *PLT1* and *PLT2* do not significantly alter auxin signaling and distribution; (2) auxins cannot induce QC and stem cell identity in *plt1 plt2* double mutants at stages where the root still contains meristematic cells; (3) ectopic PLT expression can induce ectopic root primordia which do not express the sensitive auxin-reporting *IAA2-GUS* marker and hence appear not to contain an auxin maximum. These data suggest that the promoting effect of auxin maxima on stem cell specification requires the *PLT* genes, but that *PLT*-mediated stem cell specification does not require auxin maxima, implying that the *PLT* genes act downstream of auxin accumulation. Future experiments will have to establish the molecular links between auxin accumulation, ARF action, and *PLT* transcription.

Role of the *PLETHORA* Genes in Hypocotyl and Root Identity

Ectopic expression of the *PLT1* and *PLT2* genes induces not only ectopic root stem cell formation but also transformations into root or hypocotyl identity, indicating that both *PLT* genes have the potential to transform the embryonic shoot region into more basal identities. The proposed role for *PLT1* and *PLT2* in early embryogenesis is consistent with the initial accumulation of their transcripts in the basal tier of the octant stage embryo proper, the region which will form the root and hypocotyl (Scheres et al., 1994). A hypothesis that fits well with the different subclasses of the ectopic expression lines is that different basal identities require different average levels of *PLT* expression. In this scenario, the initial expression of *PLT* in the basal domain is sufficient to specify hypocotyl identity while the more prolonged expression in the root progenitor cells confers root identity, and the continued strong expression in the incipient QC region specifies the QC and the surrounding stem cells. In line with this scenario, we have identified putative cosuppression lines of *PLT* genes which contain seedlings completely lacking hypocotyl and root. Thus, further redundancy may exist in *PLT* gene action, and mutations in yet unidentified *PLT*-related genes may reveal roles for this gene family in hypocotyl and root identity.

Experimental Procedures

Plant Lines and Growth Conditions

Arabidopsis thaliana ecotypes Wassilewskija (WS), Landsberg *erecta* (Ler), and Columbia (Col) were used. Origins and ecotypes of mutant and transgenic lines are as follows: *plt1-1* and *plt1-2* (WS), INRA T-DNA lines (Bechtold et al., 1993); *plt1-3*, *plt1-4*, and *plt2-2* (WS), the BASTA population of the *Arabidopsis* Knockout Facility (Sussman et al., 2000); *plt1-5* (L*er*), line GT7616 from the CHSL Genetrap collection (http://genetrap.cshl.org/); *plt2-1* (L*er*), line SGT4287 from the IMA insertion collection (Parinov et al., 1999); *scr-1* (WS), Scheres et al. (1995); *scr-4* (WS), Fukaki et al. (1998); *shr-1* (WS), Benfey et al. (1993); *mp-T370* (L*er*), Berleth and Jürgens (1993); *slr-1* (Col), Fukaki et al. (2002); *Cyclin-GUS* (Col), Colón-Carmona et al. (1999); QC25, QC46, and QC184 (WS), Sabatini et al. (2003); *pSHR-SHR-GFP shr-2* (Col), Nakajima et al. (2001); *DR5-GUS* (Col), Ulmasov et al. (1997). Growth conditions of seedlings and embryos are described in Sabatini et al. (1999) and Scheres et al. (1995), respectively.

Insertion Sites of the *plt* Mutants

Sequences flanking the left border (LB) of the T-DNA were determined after amplification with either Vectorette-PCR (for *plt1-1* and *plt1-2*; Morrison and Markham, 1995), TAIL-PCR (for *plt1-3*; Schomburg et al., 2003), or PCR (for *plt1-4* and *plt2-2*). The *Ds* insertion site in *plt1-5* (http://genetrap.cshl.org/) was confirmed with PCR. For *plt2-1*, the sequence flanking the 5′ region of *Ds* was amplified with PCR and sequenced. Insertion sites are as follows: *plt1-1*, −1237 bp; *plt1-2*, −5443 bp; *plt1-3*, 734 bp; *plt1-4*, 1105 bp, *plt1-5*,

733 bp; *plt2-1*, 664 bp; *plt2-2*, 1473 bp from the putative translation start point.

Linkage Analysis of *plt1-1*

When homozygous *plt1-1* plants were crossed to wild-type WS, heterozygous F1 seedlings displayed significantly weaker staining compared to homozygous seedlings. The F2 progeny segregated nonstaining plants (representing wild-type), weak staining plants (representing *plt1-1* heterozygotes), and strong staining plants (representing *plt1-1* homozygotes) in a 1:2:1 ratio (15:34:16). All plants with strong staining displayed the mutant phenotype while the rest did not, showing that the phenotype was linked to the T-DNA insertion that caused the staining pattern. Cosegregation of the T-DNA insertion with the staining pattern was demonstrated by PCR using insertion-specific primers ($n > 50$).

cDNA Isolation

PLT1 cDNA was isolated from the root cDNA library (WS) described by Wada et al. (1997) using a gene-specific probe directed to the first exon. *PLT2* cDNA containing the whole predicted coding sequence was isolated from root RNA (Col) by RT-PCR with primers cPLT2-F3 (5′-tccaattcctatcatttcagatc-3′) and cPLT2-R2 (5′-agaa gactccagccgatcct-3′).

Transgenic Plants

pGreenII vectors (Hellens et al., 2000; http://www.pgreen.ac.uk) were used for plant transformation. *pPLT1-GFP-PLT1* and *pSCR-YFP* were transformed into WS, and activator and target constructs were transformed into Col. Details for plasmid constructions are provided in the Supplemental Data online at http://www.cell.com/cgi/content/full/119/1/109/DC1/.

Marker Gene Analysis

Cyclin-GUS, *pSCR-YFP*, *DR5-GUS*, and QC markers were crossed to *plt1-4 plt2-2*. Plants homozygous for the *plt* mutations as well as for transgene markers were isolated from the F2 or F3 population and analyzed in the next generation. For SHR-GFP expression, *pSHR-SHR-GFP shr-2* was crossed to *plt1-4 plt2-2*. Plants homozygous for *plt* and *shr* mutations were isolated in the F2 and GFP was analyzed in a population segregating for *pSHR-SHR-GFP*. For *PLT1* expression analysis in *slr-1*, the *plt1-1* homozygote was crossed to Col or the *slr* homozygote and analyzed in the F1. In all experiments, parental transgenic lines were used as a control. For auxin treatments of *plt1 plt2* QC25, 2 dpg seedlings were taken from plates and cultured for 24 to 96 hr in 6 well plates containing 7 ml 0.5 × GM (0.5 × Murashige and Skoog salt mixture, 1% sucrose, and 0.5 g/l 2-[N-morpholino] ethanesulfonic acid, pH 5.8) in the absence or presence of 0.05, 0.5, 5, or 50 μM IAA or NAA.

RT-PCR Analysis

For auxin treatments, 2 dpg seedlings were taken from plates and cultured 24 and 72 hr in 250 ml erlenmeyers containing 50 ml 0.5 × GM in the absence or presence of 0.05, 0.5, 5 or 50 μM IAA or NAA. For analysis of overexpressors, seedlings were collected from 4 to 10 dpg. Details of the RT-PCR protocols are provided in the Supplemental Data on the *Cell* website.

Microscopy

For whole-mount visualization, seedlings or ovules were cleared and mounted according to Willemsen et al. (1998) or Aida et al. (1997), respectively. Starch granules and β-glucuronidase activity were visualized as described (Willemsen et al., 1998). Root length was measured as described (Willemsen et al., 1998). The number of root meristematic cells was obtained by counting cortex cells showing no signs of rapid elongation. For confocal microscopy, roots or dissected embryos were mounted in 10 μM propidium iodide or 7% glucose, respectively. Histological sections were prepared according to Scheres et al. (1994) and stained with Toluidine blue. Whole-mount in situ hybridization was performed manually using a protocol described by Friml et al. (2003a). In situ hybridization in paraffin sections was performed according to Aida et al. (2002). Riboprobes were prepared from nucleotides 1–525 or 1–560 of the *PLT1* or *PLT2* cDNA, respectively.

Acknowledgments

We thank Cold Spring Harbor Laboratory for providing the GT7616 line, Institute of Molecular Agrobiology for the SGT4287 line, P. Benfey, T. Berleth, P. Doerner, H. Fukaki, and S. Sabatini for mutant or transgenic plants, D. Weijers and R. Offringa for the *RPS5A* promoter sequence, P. Brouwer, R. Leito, M. Terlou, and W. Veenendaal for technical assistance, F. Kindt for artwork, B. Krizek for communicating unpublished results, M. Tasaka for enabling M.A. to accomplish part of this work in his laboratory, and K. Boot, H. Fukaki, M. Tasaka, P. Weisbeek, and D. Welch for helpful comments on the manuscript. M.A. was supported by a JSPS Postdoctral Fellowship for Research Abroad 2001 and a Grant for Japan-Europe Scientists Exchange Program 2001 from Novartis Foundation (Japan) for the Promotion of Science. D.B. was supported by a Greek State Scholarship Foundation (IKY) grant. The work of R.A. and Y.-S. N. was supported by the College of Agricultural and Life Sciences and the Graduate School of the University of Wisconsin and by grants (0133663 and 0209786) from the National Science Foundation. B.S. was supported by an N.W.O. PIONIER grant.

Received: March 1, 2004
Revised: August 24, 2004
Accepted: August 26, 2004
Published: September 30, 2004

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Accession Numbers

PLT1 and *PLT2* cDNA sequences were deposited in GenBank with accession numbers AY506549 and AY506550, respectively.