The PIN auxin efflux facilitator network controls growth and patterning in *Arabidopsis* roots

Ikram Blilou[1], Jian Xu[1]*, Marjolein Wildwater[1]*, Viola Willemsen[1]*, Ivan Paponov[2], Jiří Friml[3], Renze Heidstra[1], Mitsuhiro Aida[1], Klaus Palme[2] & Ben Scheres[1]

[1]*Department of Molecular Genetics, Utrecht University, Padualaan 8, 3584CH Utrecht, The Netherlands*
[2]*Institut für Biologie II , Universität Freiburg, Schänzlestrasse1, 79104, Freiburg, Germany*
[3]*Zentrum für Molekularbiologie der Pflanzen, Universität Tübingen, Auf der Morgenstelle 3, 72076 Tübingen, Germany*

* These authors contributed equally to this work

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Local accumulation of the plant growth regulator auxin mediates pattern formation in *Arabidopsis* roots and influences outgrowth and development of lateral root- and shoot-derived primordia. However, it has remained unclear how auxin can simultaneously regulate patterning and organ outgrowth and how its distribution is stabilized in a primordium-specific manner. Here we show that five *PIN* genes collectively control auxin distribution to regulate cell division and cell expansion in the primary root. Furthermore, the joint action of these genes has an important role in pattern formation by focusing the auxin maximum and restricting the expression domain of *PLETHORA* (*PLT*) genes, major determinants for root stem cell specification. In turn, *PLT* genes are required for *PIN* gene transcription to stabilize the auxin maximum at the distal root tip. Our data reveal an interaction network of auxin transport facilitators and root fate determinants that control patterning and growth of the root primordium.

In *Arabidopsis* root development, a distal auxin maximum correlates with pattern formation and the orientation and extent of cell division[1]. Inhibition of polar auxin transport strongly affects these processes[1]. The initiation of lateral roots and leaf primordia is also associated with changes in auxin transport[2–4]. These observations point to polar auxin transport as a major factor in organ formation. Recent analyses of the *Arabidopsis* PIN proteins revealed their association with auxin maxima in distal domains of organ primordia[2–4]. The *PIN* genes are thought to encode components of the auxin efflux machinery mediating polar auxin transport, as concluded from the polar localization of PIN membrane proteins and auxin uptake experiments[5–11]. PIN proteins might participate directly in auxin transport or help in the assembly of other proteins with efflux activity such as the AtMDR/PGP proteins[12,13]. Mutations in the ARF-GEF GNOM, required for vesicle transport of PIN1, also lead to developmental defects that resemble those caused by interfering with auxin transport[14–16].

All single *pin* mutants described so far display different weak phenotypes in primary roots[4–8], in contrast with *gnom* mutants and polar auxin transport inhibitor treatments. Here, we investigate the collective contribution of five *PIN* genes in the control of cell division and cell expansion during root outgrowth. Furthermore, we assess how the *PLETHORA* (*PLT*) genes, which encode auxin-inducible AP2-domain putative transcription factors necessary and sufficient for stem cell specification in the root primordium[17], respond to and regulate *PIN* gene activity during pattern formation.

PIN protein localization is changed in *pin* mutants

The PIN proteins described so far are expressed in specific but overlapping regions of the root meristem[6–8]. PIN1 mainly resides at the basal end of the vascular cells[8] but weak PIN1 signals can be detected in the epidermis and the cortex (Fig. 1b). PIN2 localizes apically in epidermal and lateral root cap cells and predominantly basally in cortical cells[6] (Fig. 1e, f). PIN3 is expressed without pronounced polarity in tiers two and three of the columella cells, at

the basal side of vascular cells and to the lateral side of pericycle cells of the elongation zone[7] (Fig. 1h, i). PIN4 is detected around the quiescent centre and cells surrounding it, and localizes basally in provascular cells[8] (Fig. 1k). PIN7 resides at lateral and basal membranes of provascular cells in the meristem and elongation zone, whereas in the columella cells it coincides with the PIN3 domain (Fig. 1j).

Auxin distribution appears to be altered in *pin* mutants[1,8] and differences in auxin homeostasis affect PIN2 expression[18]. We therefore determined whether *pin* mutants have altered expression of remaining PIN proteins. In *pin3pin4pin7*, enhanced PIN1 protein was detected in lateral-basal membranes of the endodermis (Fig. 1c, d). Moreover, ectopic PIN2 protein was detected at the basal end of provascular cells that normally express PIN3 and PIN7 (Fig. 1g). PIN4 expression expands to tier three of the columella cells in *pin3* single mutants and *pin2pin3* double mutants with a membrane localization similar to PIN3 in the wild type (Fig. 1l and data not shown). In *pin3pin7*, PIN4 expands to the lateral root cap (Fig. 1m).

Our data show that defects in *pin* mutants can be masked by ectopic activity of the remaining *PIN* genes. A comprehensive mutant analysis is therefore necessary to uncover full gene function. To this end, we generated all mutant combinations for *PIN1*, *PIN2*, *PIN3*, *PIN4* and *PIN7*, which group together within the eight-member *PIN* gene family. We verified phenotypes in independent allelic combinations to exclude influences of background modifiers (Supplementary Table 1).

PIN genes control cell division zone size in the root meristem

Classical experiments revealed that externally added auxins can stimulate cell division[19] but it is unknown whether internal auxin distribution regulates cell division in primordia. *PIN* genes are required for outgrowth of all organs[2,3,20], and we assessed whether this reflected their contribution to the *in vivo* regulation of cell division. In roots, oriented cell divisions accompanied by a low rate of cell expansion occur in the distal meristem zone (Fig. 2A).

pin1 and *pin2* single mutants display a slight reduction of root

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length and root meristem size (Supplementary Fig. 2) whereas *pin3*, *pin4* and *pin7* single mutants only display subtle division defects in the quiescent centre and columella root cap[8] (Supplementary Fig. 1d–h). Most double-mutant combinations show additive defects in orientation of cell division, root length and root meristem size (Supplementary Figs 1 and 2). However, *pin1pin2* and all triple and quadruple mutants containing *pin2* show more-than-additive reduction in root size and root meristem size (Fig. 2B, C and Supplementary Fig. 2a, b), suggesting that PIN2 plays a pivotal role in cell division control. The *pin1pin2* double mutant suggests that the role of PIN2 in meristem size control is masked in the *pin2* single mutant mainly by the activity of PIN1 in the PIN2 domain (Fig. 1b). PIN2 is a main component for mediating proximal (basipetal) auxin transport (Fig. 1e, f, n), which implies that basipetal transport to meristematic cells has a critical role in meristem length regulation. Consistent with the notion of auxin shortage in the meristem

zone, treatment with auxins restored meristem size of *pin1pin2* and *pin2pin3pin7* to that of wild type (data not shown). Our data substantiate physiological evidence indicating a role for basipetal auxin transport in root growth[21,22] and identify control of cell division as a major factor in this process.

The extreme reduction in root meristem size in *pin2* mutant combinations is reminiscent of the phenotype obtained upon diphtheria toxin-mediated genetic ablation of root cap cells[23]. In such plants we found high *DR5::GUS* accumulation in the pro-vascular tissue (Fig. 2E, F), supporting the notion that lateral auxin redistribution does not occur in the absence of columella cells with laterally oriented PIN proteins.

Our data imply that basipetal transport and lateral redistribution of auxin are both critical for maintenance of the meristem zone. A potential mechanism to transport auxin to every cell in the meristem is indicated by PIN2 localization at basal membranes of cortex cells (Fig. 1e, f) and the expression domains of vascular PIN3 and PIN7, which suggest an 'auxin reflux' loop (Fig. 1h, j, n). We investigated auxin transport in the root by expressing the bacterial auxin biosynthetic enzyme IAAH under the WOX5 (ref. 24) promoter, which allows induction of auxin biosynthesis in the quiescent centre (Fig. 2G) by external addition of the precursor IAM[25]. After different time points of induction, we monitored auxin accumulation using the *DR5-GFP* reporter. In line with the observed polar orientation of the PIN proteins, enhanced auxin responses appear first in the columella region, subsequently in the lateral root cap and then in the epidermis; enhanced responses in provascular strands only emerge at later stages (Fig. 2H, a–d). Epidermal and provascular auxin responses could be reduced by the polar transport inhibitor N-naphthylphthalamic acid (NPA), suggesting that accumulation of newly synthesized auxin in these regions is due to an NPA-sensitive auxin transport loop (Fig. 2H, e). In *pin2* mutant background the induced responses are restricted to the lateral root cap and epidermis, confirming a role for basipetal transport of auxin towards provascular cells in the root meristem (Supplementary Fig. 3).

Our results suggest that the capacity to circulate auxin through loop-oriented PIN efflux facilitators regulates meristem size. Such a loop system can redeploy auxin and hence operate at least partially independently from shoot auxin supply, consistent with the ability of isolated root systems to maintain growth without external auxin application. In a wider perspective, these data support that PIN protein localization predicts auxin transport routes.

PIN genes regulate cell expansion and root elongation zone size

The capacity to stimulate cell expansion upon external application has been a defining property for auxins[19]. Recent analysis of tropic responses revealed that *PIN* genes play a part in differential auxin distribution, which is accompanied by differential cell expansion[7,9,11,26]. It is, however, unknown whether *PIN* genes also regulate general cell expansion during organ growth. In roots, rapid cell expansion without cell division occurs in the elongation zone located proximal to the meristem zone (Fig. 2A).

Final cell size is affected in several *pin* mutants, but no additive effects occur in mutant combinations, suggesting that *PIN* gene action on cell expansion is complex. Nevertheless, in single allelic combinations of *pin1pin3* and *pin3pin7*, the change in final cell length is the major factor accounting for the reduction in root length (Supplementary Fig 2, red fonts). In contrast to mature cell size, the size of cells immediately after departure from the meristem is not affected in any *pin* mutant combination (Supplementary Fig. 2).

We observed a reduction in the size of the elongation zone in *pin* mutant combinations, which mostly correlated well with reduction in meristem size. However, in *pin3pin4pin7* only the elongation zone size is reduced (Supplementary Fig. 2, blue fonts). These data
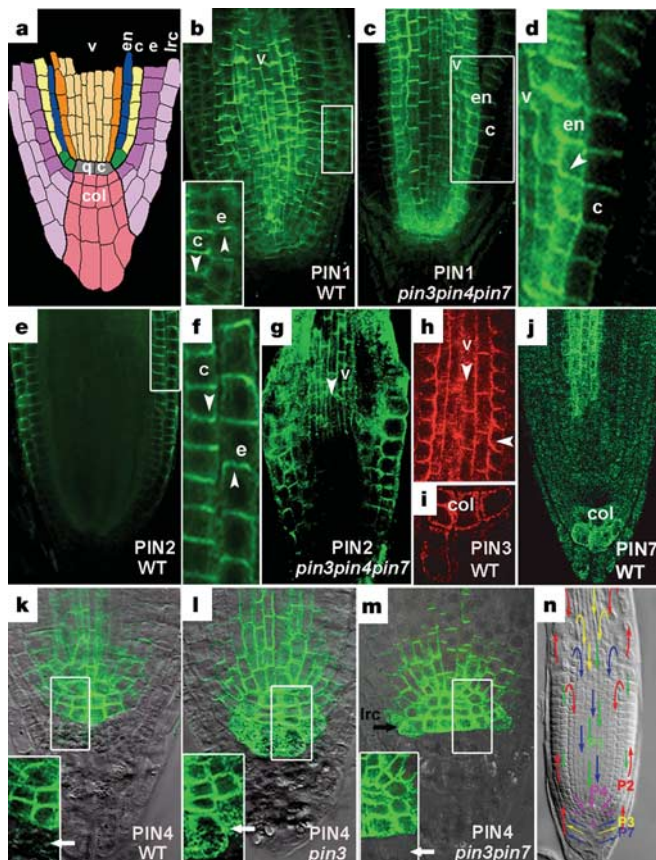
Figure 1 PIN expression and protein localization in roots of wild-type (WT) and *pin* mutant *Arabidopsis*. **a**, *Arabidopsis* root meristem with columella (col), quiescent centre (qc), lateral root cap (lrc), epidermis (e), cortex (c), endodermis (en) and vascular bundle (v). **b–d**, PIN1 immunolocalization: **b**, wild type; inset shows boxed area enlarged. Arrowheads depict polar localization. **c**, **d**, *pin3pin4pin7* (Allelic Combination 1(AC1)); boxed area in **c** is enlarged in **d**. Arrowheads in **d**, PIN1 upregulation in the endodermis. **e–g**, PIN2 protein: **e**, **f**, Wild type; boxed area in **e** is enlarged in **f**. Arrowheads in **f**, apical PIN2 in the epidermis and basal in the cortex. **g**, *pin3pin4pin7* (AC1); arrowhead shows ectopic expression. **h**, **i**, PIN3 protein in wild-type root meristem. Arrowheads in **h**, PIN3 in the pericycle. **j**, PIN7–green fluorescent protein (GFP) fusion. **k–m**, PIN4 protein: **k**, wild type; white arrow, wild-type differentiated columella cell without PIN4. **l**, *pin3* (white arrow); **m**, *pin3pin7* (AC1): black arrow, PIN4 expansion to the lateral root cap; white arrow as in **k**. Insets: enlargements of boxed areas showing details of PIN4 localization. **n**, Localization of PIN proteins suggests auxin transport routes. PIN1 (green), PIN2 (red), PIN3 (yellow), PIN4 (violet) and PIN7 (blue). Immunolocalization signals are green in **b–g** and **k–m** and red in **h**, **i**.

indicate that the region where cell elongation occurs can be independently controlled by *PIN* gene activity.

Together, our data reveal that modulation of PIN activities can separately affect meristem size, elongation zone size and final cell size. These effects are not additive but probably result from interactions between changes in auxin distribution and transcriptional or translational responses influencing carrier components. We conclude that PIN-mediated modulation of auxin distribution controls both cell division and cell elongation and thereby contributes to the 'organizing' role of auxin in organ growth.

PIN genes regulate *PLT* expression and pattern the distal root meristem

Polar auxin transport is a major contributor to root meristem patterning in *Arabidopsis*, and the specification of distal cell types correlates well with the auxin response maximum[1]. Close to the auxin maximum, the quiescent centre maintains surrounding cells as stem cells (Fig. 1a). Quiescent centre and stem cell specification require SHR and SCR, putative GRAS family transcription factors[27,28] and the redundantly acting PLT1 and PLT2 AP2-domain putative transcription factors[17]. *PLT* transcript accumulation is
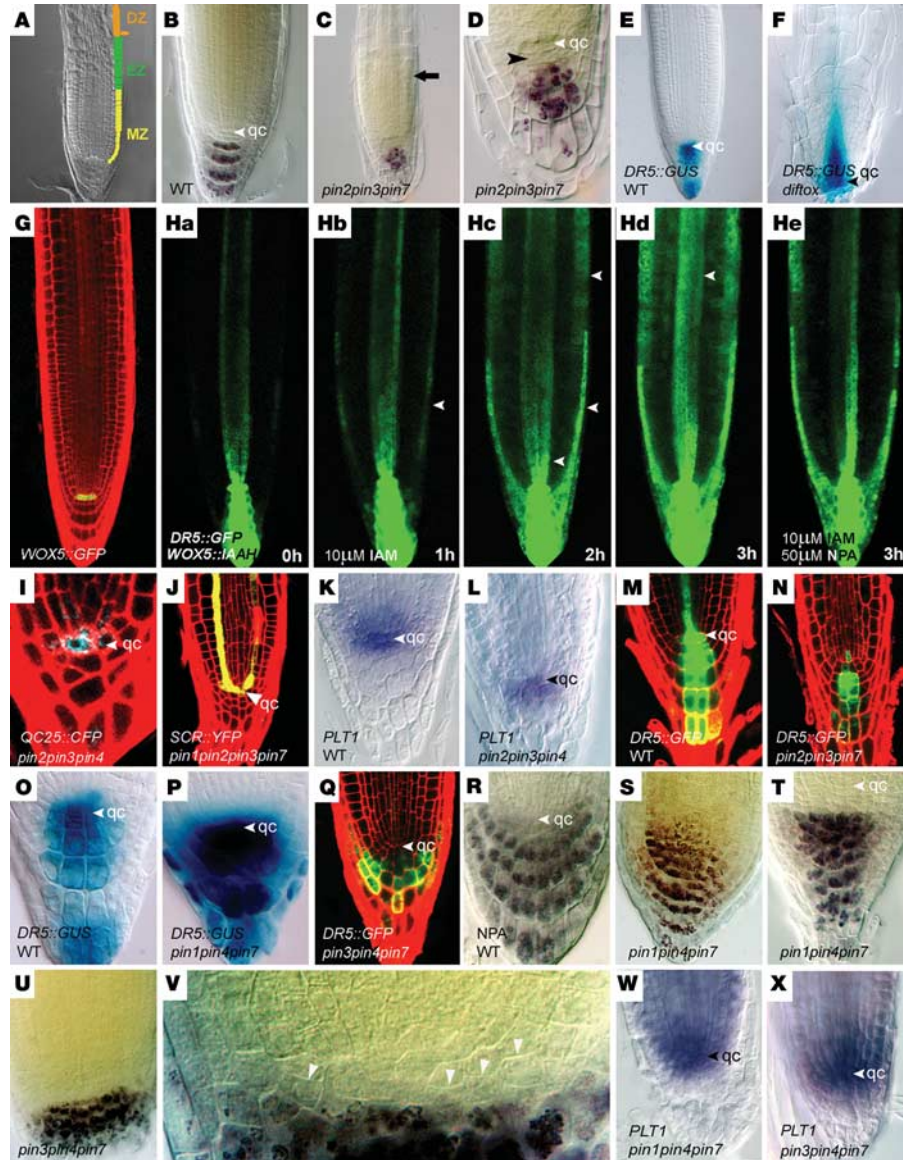
Figure 2 *PIN* genes control meristem size and patterning in *Arabidopsis* roots. **A–F**, Meristem size control. **A**, Wild-type meristem zone (MZ), elongation zone (EZ) and differentiation zone (DZ). **B**, Columella cell staining. **C**, **D**, *pin2pin3pin7* [(AC1)]: arrow in **C**, border of MZ and EZ; black arrowhead in **D**, columella stem cells. **E**, **F**, *DR5::GUS* expression in wild type (**E**) and *pRCP1::DT-A* [tsM] (root cap expressed diphtheria toxin, 'diftox') (**F**). **G**, *WOX5* promoter specificity for quiescent centre. **H**, **a–e**, *DR5::GFP* in *WOX5::IAAH* plants. **a**, Control. **b–d**, *DR5::GFP* upregulation (arrowheads) after IAM application. **e**, No provascular upregulation on 50 μM NPA. **I–L**, Patterning in *pin2* mutant combinations. **I**, *QC25::CFP* (cyan fluorescent protein) in *pin2pin3pin4* [(AC2)]. **J**, *SCR::YFP* (yellow fluorescent protein) in *pin1pin2pin3pin7* [(AC1)]. **K**, **L**, In wild type (**K**) and in *pin2pin3pin4* [(AC2)] (**L**), *PLT1* transcript is restricted to the quiescent centre and stem cells.

M, **N**, *DR5::GFP* in wild type (**M**) and *pin2pin3pin7* [(AC1)] (**N**). **O–X**, *pin* mutants with patterning defects. Wild type treated with NPA (**R**). In 33% of *pin1pin4pin7* [(AC1)] *DR5::GUS* (**P**), starch-granule-containing columella cells (**S**) and *PLT1* expression (**W**) expand proximally (white arrowhead). In *pin1pin4pin7* [(AC2)], columella cells also expand laterally (**T**). In *pin3pin4pin7* [(AC1)], mature columella cells (**U**), their stem cells (arrowheads in **V**), *DR5::GFP* expression (**Q**) and *PLT1* mRNA (**X**) expand laterally. *PLT1* transcript: whole-mount *in situ* hybridization (blue/purple signals). Differentiated columella cells: starch granule staining (purple). *DR5::GUS*, blue. GFP fluorescence, green. qc, quiescent centre. **A–F**, **K**, **L**, **O**, **P**, **R–X**, Nomarski optics; **G**, **H**, **I**, **J**, **M**, **N**, **Q**, CLSM after propidium iodide staining.

correlated with auxin accumulation in embryos and primary and lateral roots, and depends on auxin response factors[17]. The identification of these critical factors for specification of distal cell types as well as the highly organized PIN gene expression domains in the

root primordium set the stage for an analysis of the role of *PIN* genes in pattern formation.

In the previously discussed *pin2* mutant combinations, meristem size is affected but distal patterning is normal, as judged by the presence of columella stem cells (Fig. 2D), the *QC25* marker for the quiescent centre (Fig. 2I), *SCR* promoter activity (Fig. 2J), *PLT1* (ref. 17) transcript distribution (Fig. 2K, L) and the DR5 auxin response marker (Fig. 2M, N).

In *pin1pin4pin7*, however, the auxin response maximum and the starch granules that mark the differentiated columella cells shift proximally in 40% of roots (Fig. 2P, S). In *pin3pin4pin7*, the auxin response maximum shifts laterally (Fig. 2Q), associated with inappropriate lateral up-regulation of PIN1 (Fig. 1c, d). Correlated with this lateral expansion, a broadening of the columella domain including its stem cells occurs (Fig. 2U, V). Consistent with changes in distal patterning, *PLT1* messenger RNA shifts proximally in *pin1pin4pin7* (Fig. 2W) and expands laterally in *pin3pin4pin7* (Fig. 2X).

The phenotypes of both triple mutants resemble effects of treatment with inhibitors of polar auxin transport (Fig. 2R) and support a role for PIN proteins in focusing and stabilizing an auxin maximum in both proximo-distal and lateral dimensions. Our data strongly suggest that the PIN proteins in this way focus expression of the auxin-inducible *PLT* genes in the distal root region, which specifies the position of the quiescent centre and stem cells.

PIN genes restrict PLT mRNA and root identity to the basal embryo pole

We addressed whether *PIN* genes control *PLT* expression and root stem cell patterning during embryogenesis. Distal quiescent centre and columella cells originate from daughter cells of the hypophysis at the early globular stage of embryogenesis (Fig. 3a–d). At that stage an auxin perception maximum is detected in the hypophysis[4].
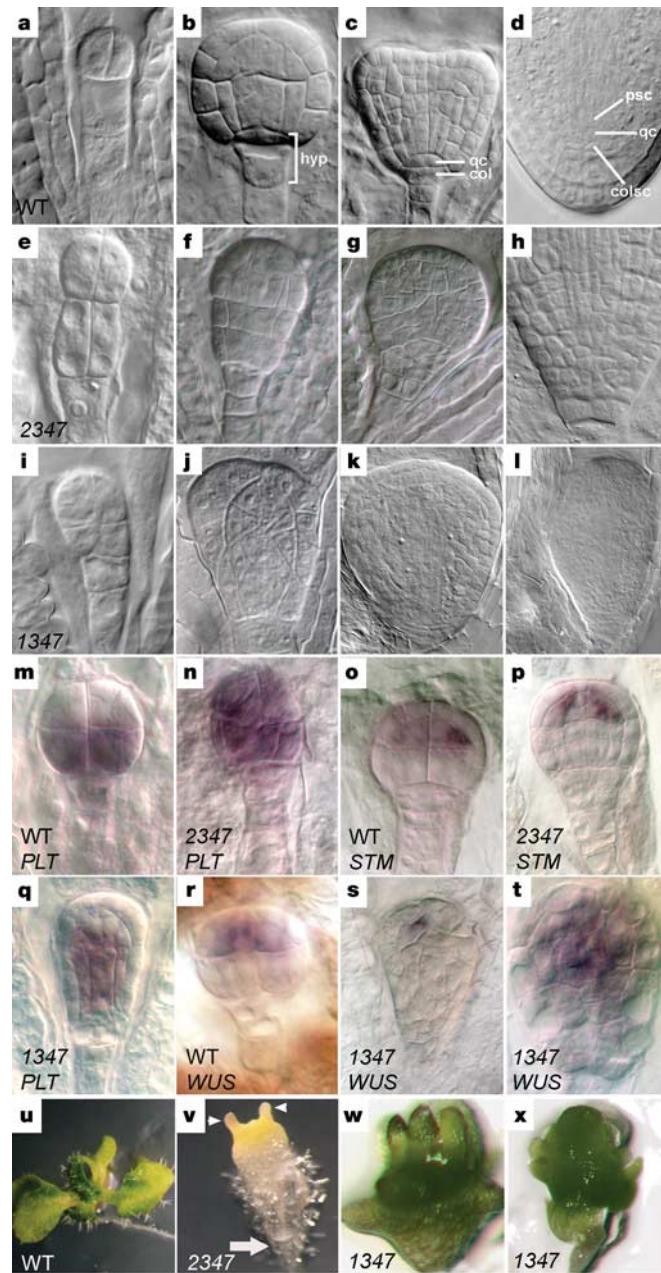
Figure 3 *PIN* genes and embryonic patterning. **a–d**, Wild-type embryo at 2-cell (**a**) globular (**b**) early heart (**c**) and torpedo stage (**d**). hyp, hypophysis; qc, quiescent centre; col, columella; sc, stem cells; psc, provascular stem cells. **e–h**, *pin2pin3pin4pin7* [(AC1)] embryos: **e**, aberrant divisions in basal cells at octant stage; **f**, basal cell duplication at globular stage; **g**, **h**, quiescent centre and stem cell division defects at heart (**g**) and torpedo stages (**h**). **i–l**, *pin1pin3pin4pin7* [(AC1)] embryos with abnormal basal (**i**) and apical cell divisions (**j–l**). **m–t**, Gene expression in *pin* quadruples: **m**, *PLT1* mRNA in wild-type preglobular embryos is restricted to basal cells; **n**, ubiquitous *PLT1* in *pin2pin3pin4pin7* [(AC1)] proembryos; **p**, **o**, *STM* in *pin2pin3pin4pin7* [(AC1)] (**p**) and wild type (**o**); **q**, *PLT1* in *pin1pin3pin4pin7* [(AC1)]; **r–t**, In comparison to wild type (**r**), *WUS* is slightly reduced (**s**) or expands (**t**). **u–x**, Explanted embryos: **u**, wild type develops normal seedlings; **v**, *pin2pin3pin4pin7* [(AC1)] develops reduced cotyledons (arrowheads) and ectopic root hairs (arrow); **w**, **x**, *pin1pin3pin4pin7* [(AC1)] explants have reduced root development and ectopic shoot-like structures at the apex (green tissues). *PLT1*, *STM* and *WUS* transcripts: *in situ* hybridization (purple). Images: Nomarski optics.
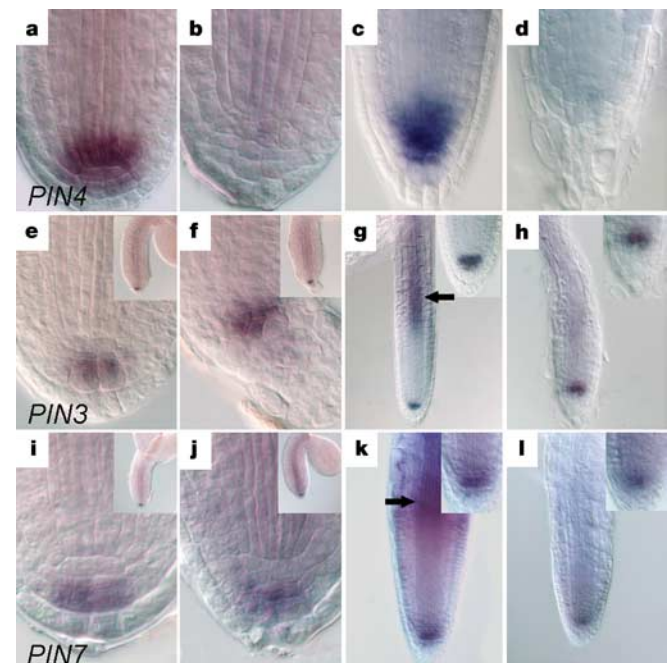
Figure 4 *PLT* genes regulate *PIN* transcript levels. **a–d**, *PIN4* mRNA localization. *PIN4* transcript in the quiescent centre, stem cells and provascular initials of wild-type embryo (**a**) and seedlings (**c**). No detectable transcripts in *plt1plt2* double-mutant embryos (**b**) and seedlings (**d**). **e–l**, *PIN3* and *PIN7* mRNA in columella cells and provascular region of wild-type embryo (**e**, **i**) and seedlings (**g**, **k**, arrows). *plt1plt2* with normal levels in embryonic columella (**f**, **j**) and seedlings (**h**, **l**). Reduction of provascular expression in seedlings (**h**, **l**). Images: Nomarski optics.

Clonal analysis and stereotyped cell division patterns show that the proximal stem cells are recruited around mid-heart stage (Fig. 3c)[29].

We found no penetrant embryonic defects in single, double and triple *pin* mutants and in *pin1pin2pin3pin7* and *pin1pin2pin3pin4*. However, in lines segregating *pin2pin3pin4pin7* and *pin1pin3pin4-pin7* mutants, marked changes were observed in embryonic division patterns and gene expression (Fig. 3e–t and Supplementary Table 2). *pin2pin3pin4pin7* mutant embryos exhibit cell division defects mainly in the basal region (Fig. 3e–h) and ~30% produce viable seedlings. In contrast, *pin1pin3pin4pin7* mutants are embryo lethal and cell division defects occur in apical and basal embryo regions[4] (Fig. 3i–l). The significance of the altered cell divisions in both quadruple-mutant embryos in terms of patterning was investigated by locating the expression domain of marker genes for root and shoot identity. *PLT1* transcript marks the basal domain of the octant stage embryo (Fig. 3m) and restricts to the quiescent centre and stem cell domain before mid-heart stage[17]. *STM* and *WUS* are required for shoot meristem function and are transcribed in shoot meristem precursor cells from early embryogenesis onwards[30,31].

pin2pin3pin4pin7 mutants contain both aberrant cell divisions and high levels of *PLT1* transcript throughout the embryo from the 16-cell stage onwards (Fig. 3n). In contrast, *PLT1* mRNA is correctly excluded from the apical region in *pin1pin3pin4pin7* mutants (Fig. 3q). Interestingly, the mRNA localization of *WUS* and *STM* does not change in *pin2pin3pin4pin7* mutants (Fig. 3o, p), whereas in *pin1pin3pin4pin7*, *WUS* transcripts are either slightly reduced (Fig. 3s) or expanded in apical embryonic cells (Fig. 3t).

In explanted wild-type embryos, the shoot and root apical meristems develop normally (Fig. 3u). In ~40% of *pin2pin3pin4-pin7* mutants, the explants develop reduced cotyledons and root hairs emerge at more apical positions (Fig. 3v and Supplementary Table 2). In ~80% of *pin1pin3pin4pin7* mutants, the explants completely arrest root growth and expand the shoot domain (Fig. 3w, x and Supplementary Table 2). The explant phenotypes of both mutants match the observed expansion of *PLT1* and *WUS* domains, because ectopic expression of these genes promotes root and shoot identity, respectively[17,32]. *PLT* gene expression is strongly dependent on the joint action of PIN proteins, in line with its dependence on auxin response factors and the correlation between *PLT* expression and auxin accumulation[17]. Most probably the *PLT* expression domain is regulated by PIN4 and PIN7, which are appropriately positioned for basal auxin transport at the pre-globular stage[4]. PIN3 and PIN2 are not expressed at this stage but ectopic PIN2 mRNA can be detected in *pin3pin4pin7* siliques (data not shown), suggesting that ectopic expression of PIN members in embryos provides a remarkably versatile compensatory mechanism for the loss of PIN4 and PIN7.

PIN-regulated early *PLT*, *WUS* and *STM* transcription suggests that auxin transport in the embryo regulates the proper expression of critical root and shoot stem cell regulators. The notion that early cellular asymmetries in membrane localization of PIN proteins are translated into the patterning of embryonic stem cell domains via regulation of auxin flux provides a conceptual framework for initial events in plant embryogenesis.

PLT genes regulate *PIN* gene expression in the root meristem

The *PLT* genes are required for specification of the stem cell niche and convey root identity when ectopically expressed[17]. Therefore we asked whether the PLT proteins could regulate the root-specific distribution of *PIN* transcripts, thereby fine-tuning the position of the stem-cell-associated auxin maximum, and cell division and cell expansion domains. Remarkably, *PIN4* transcript, which overlaps with the *PLT* transcripts in wild type, is undetectable in 94% and aberrant in 6% of the embryos and seedlings of *plt1plt2* (Fig. 4a–d). *PIN3* and *PIN7* transcripts are normal in columella cells (Fig. 4e–l) but are markedly reduced in the provascular domain of the post-embryonic root elongation zone (Fig. 4g, h, k, l). Thus, the *PLT* genes control *PIN* mRNA distribution.

Discussion

Our findings suggest an elegant mechanism for embryonic root primordium formation and stabilization. In our model, PIN proteins restrict *PLT* expression in the basal embryo region to initiate root primordium formation (Fig. 5a). In turn, *PLT* genes maintain *PIN* transcription, which stabilizes the position of the distal stem cell niche (Fig. 5b). At a distance from the auxin maximum, *PLT* genes maintain PIN3 and PIN7, which reinforce provascular acro-petal auxin flux. In this way a 'reflux' loop is created that controls auxin distribution in the growing primordium and meristem (Fig. 5b, c). The loop stabilizes the auxin maximum and the PLT-dependent stem cell domain in the distal root tip. Moreover, it localizes meristem and cell expansion zones in the proximal meristem and regulates final cell size (Fig. 5c).

Reporter genes and direct auxin measurements are consistent with the presence of a transport-regulated auxin gradient in the root meristem[1], but local auxin biosynthesis and catabolism may also contribute to the auxin concentration profile[33]. Furthermore, auxin response may be regulated differently in the different root zones by processes such as differential SCF[TIR1]-mediated proteolysis[34]. □
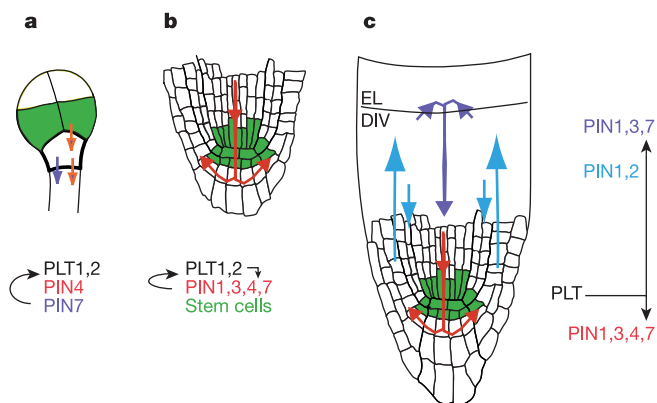
Methods

Materials

Figure 5 Model for primordium formation by PIN–PLT interactions. **a**, PIN-mediated root primordium specification by restriction of PLT transcripts in octant/16-cell embryo stage. **b**, At later stages of embryogenesis, PIN action further restricts *PLT* transcripts to define the stem cell region and *PLT* genes start controlling root-specific *PIN* gene expression. **c**, In post-embryonic roots, PIN-mediated auxin transport stabilizes the stem cell region and regulates cell division (DIV) in the meristem zone and cell expansion in the elongation zone (EL). *PLT* genes control several members of the *PIN* gene family to generate primordium-specific auxin distribution.

ovules were dissected and embryos were prepared as described in ref. 29. Embryo explants were cultured on petri dishes containing one-half GM medium and grown on the same medium for 4 weeks. For exogenous auxin application, wild type and multiple mutants were grown for 4 days (d) and transferred to medium supplemented with 100–200 nM NAA for another 3 d. Induction of auxin biosynthesis was performed by transferring plants to medium containing 10 µM of the precursor IAM for 0–3 h. Images were taken on a Zeiss Axioskop with a Nikon DXM1200 digital camera.

In situ hybridization and immunolocalization

For whole-mount *in situ* hybridization[8], gene-specific 600-base-pair complementary DNA fragments for *PIN4*, *PIN3* and *PIN7* were used as probes. *PLT1* probe was described in ref. 17 and *STM* and *WUS* probes were synthesized using the complete cDNA sequence. Immunolocalizations were performed on whole-mount roots of 5–7-day-old seedlings[7,37]. PINs antibodies and reagents were described in refs 5–8. Imaging was performed using a Leica SP2 inverted confocal microscope and accompanying software.

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