The Plant Cell, Vol. 9, 1713–1725, October 1997 © 1997 American Society of Plant Physiologists

Promoter Trap Markers Differentiate Structural and Positional Components of Polar Development in Arabidopsis

Jennifer F. Topping and Keith Lindsey[1]

Department of Biological Sciences, University of Durham, South Road, Durham DH1 3LE, United Kingdom

To investigate mechanisms involved in establishing polar organization in Arabidopsis embryos and seedlings, we used promoter trapping to identify molecular markers (β-glucuronidase fusion genes) expressed in spatially restricted patterns along the apical–basal axis. Three markers were identified that are expressed, respectively, in the embryonic and seedling root tip (*POLARIS*), cotyledons and shoot and root apices (*EXORDIUM*), and root cap (*COLUMELLA*). Each marker was crossed into the mutants *hydra* and *emb30*, which are defective in embryonic and seedling morphogenesis. All three markers were expressed in *hydra* mutants in patterns similar to those observed in phenotypically wild-type embryos and seedlings. In *emb30* mutants, the *EXORDIUM* marker was expressed in cotyledons but not in the expected position of shoot and root meristems, and the marker *COLUMELLA* was not expressed at all, which is consistent with the view that the *emb30* mutant, but not *hydra*, lacks shoot and root meristems. However, *POLARIS* was expressed in the basal part of *hydra* embryos lacking an embryonic root and in the basal parts of both *hydra* and *emb30* seedlings. Expression of *POLARIS* is inducible by exogenous auxin and suppressed by cytokinin but is unaffected by inhibitors of polar auxin transport or cell division. We conclude that *POLARIS* differentiates positional aspects of polar development from structural aspects.

INTRODUCTION

The establishment of polarity is a common feature in the development of multicellular organisms and is evident both during cell differentiation and as a component of supracellular organization. In both higher and many lower plants, a fundamental feature of supracellular polarity is represented by organization in the apical–basal plane, whereby the relative positions of the shoot apex, the plant body, and the root apex are constant. Furthermore, polarity may play a role in the generation of cell diversity. It is known that the products of certain cell divisions have different fates. For example, the zygote of Fucus divides to produce progenitor cells of the thallus and rhizoid, respectively (Quatrano et al., 1991); the immediate division products of the zygote of Arabidopsis represent progenitors of the embryo proper and suspensor (Mansfield and Briarty, 1991), and the products of microspore mitosis become the vegetative and generative cells of the pollen grain (Terasaka and Niitsu, 1987). In the latter example, the asymmetric division of the microspore appears to play a determinative role in the expression of daughter cell–specific gene expression patterns (Eady et al., 1995).

One interpretation of these observations is that the asymmetrical distribution of regulatory molecules may be critical or determinative in the control of cell fate in plants. However, the molecular mechanisms that provide positional information during vegetative plant development are largely obscure, and we have very little information on the molecular processes associated with the determination of supracellular polarity (although insight into mechanisms regulating floral organ position and identity has improved dramatically in recent years; Coen and Meyerowitz, 1991; Coen et al., 1995). In animal systems, such as Drosophila, it is known that the asymmetric distribution of transcription factors and other signaling molecules establishes polarity and subsequently cell fate in the developing embryo (Kornberg and Tabata, 1993; González-Reyes et al., 1995). One approach to address the molecular basis of the regulation of polar development in plants has been to identify mutants in which the establishment of polarity is defective and thereby identify the genes that are essential to this process (Mayer et al., 1991). A complementary approach would be to identify molecular markers that define components of the pathway of polar development and that can be used to investigate the organization of gene expression in anatomically defined mutants defective in aspects of morphogenesis such as polar organization.

We have previously described the production and screening of transgenic plants containing a promoter trap that can generate cell type–specific markers (Topping et al., 1991, 1994; Lindsey et al., 1993; Wei et al., 1997). We describe the use of three such markers, expressed embryonically and/or postembryonically in Arabidopsis, to investigate aspects of

[1]To whom correspondence should be addressed. E-mail Keith.Lindsey@durham.ac.uk; fax 44-191-374-2417.

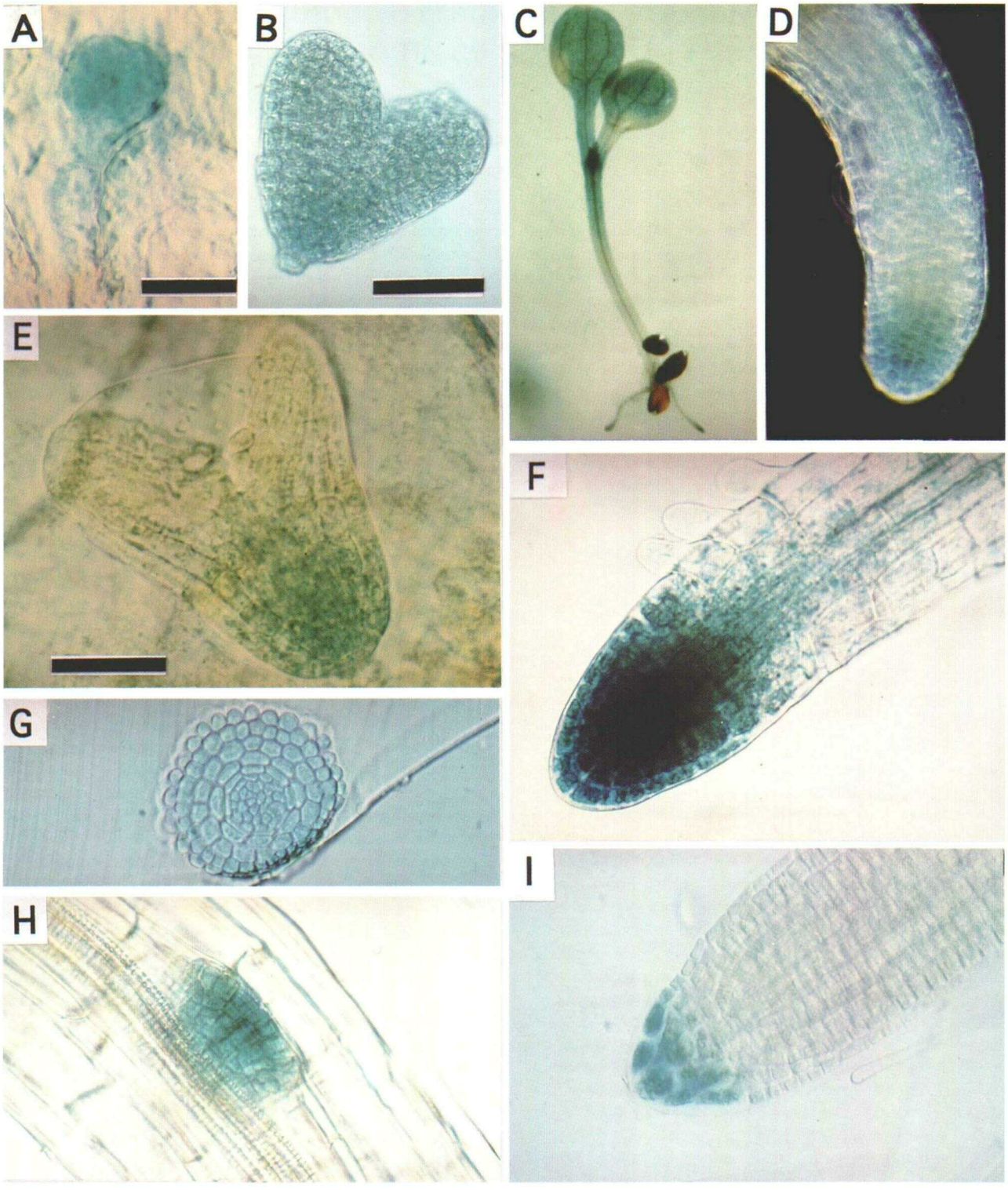

Figure 1. Expression of *EXO*, *PLS*, and *COL* in Phenotypically Wild-Type Arabidopsis.

polar development in two mutants exhibiting dramatic defects in morphogenesis. One mutant, *emb30*, is characterized in part by a failure to develop shoot and root meristems, and it has been considered defective in the establishment of polar organization (Mayer et al., 1993). A second mutant, *hydra*, is defective in the establishment of an embryonic root (Lindsey et al., 1996; Topping et al., 1997). By analyzing marker gene activities in each mutant, it has been possible to identify a molecular marker, *POLARIS* (*PLS*), that can distinguish between two aspects of root organogenesis, namely, cytodifferentiation and cell positioning. We provide evidence that the expression of *PLS* is independent of root meristem formation or activity but is linked to the position of root development, thereby representing a marker of embryonic and seedling polarity. Expression is induced by exogenous auxin and suppressed by cytokinin, and we propose a model to describe the relationship of root positioning or potentiation, meristem organization, and hormonal interactions in the developing Arabidopsis root.

RESULTS

Molecular Markers of Cell Types

To identify novel molecular markers that might prove useful in dissecting pathways of axial development in plants, a transgenic population of Arabidopsis containing a promoter trap T-DNA from pΔgusBin19 (Topping et al., 1991) was screened for lines showing β-glucuronidase (*gus*) fusion gene expression in a range of embryonic and postembryonic cell types (Lindsey et al., 1993; Topping et al., 1994).

Three transgenic marker lines exhibiting GUS activity in spatially restricted patterns were chosen for further analysis. Line AtEM201 exhibited GUS fusion activity constitutively during embryogenesis, from the octant stage onward, and in the cotyledons, shoot apex, and primary and lateral root apices of seedlings (Figures 1A to 1D). This marker has been termed *EXORDIUM* (*EXO*). Line AtEM101 exhibited GUS fusion activity in the basal half of the embryo from the heart stage onward (Figure 1E). These cells contribute to the seedling hypocotyl and root (Scheres et al., 1994). Activity, however, was strongest in the region of the developing embryonic root tip. In AtEM101 seedlings, GUS expression was found faintly in the seedling hypocotyl but most strongly in the seedling and mature plant root tip, including the lateral root tip from early stages of pericycle division (Figures 1F and 1H). Transverse sections of AtEM101 mature seedling root tips showed GUS fusion activity in a collection of cell types (columella and lateral root cap, epidermis, meristem, and immature vascular tissues), which have in common their position at the root tip rather than a common lineage (Figure 1G; Dolan et al., 1993; Scheres et al., 1994). This marker has been termed *PLS*. Marker line AtBFM117 had GUS activity specifically in the root cap of both primary and lateral roots (Figure 1I), and the marker has been termed *COLUMELLA* (*COL*). Lines AtEM101 and AtEM201 have each been shown to contain a single copy of the promoter trap T-DNA, and each of the tagged genes has been found to be expressed as a fusion transcript between the respective native gene and *gusA* (Topping et al., 1994). In line AtBFM117, there are probably three T-DNA copies present (J.F. Topping, D. Worrall, and K. Lindsey, unpublished data). Because of the specificity of GUS activity, it seems likely that only one *gus* transgene is functional under our experimental conditions.

Expression of Marker Gene Fusions in Mutant Backgrounds

To investigate the expression patterns of the markers in different mutant backgrounds, we introduced each by crossing into two mutants defective in apical–basal development.

Mutants

Two transgenic lines were identified that showed segregating mutant phenotypes. One was designated *hydra*. This mutant shows some morphological similarity to the *fass* mutant (Torres-Ruiz and Jürgens, 1994), but complementation

Figure 1. (continued).

(A) to **(D)** show *EXO* expression, **(E)** to **(H)** show *PLS* expression, and **(I)** shows *COL* expression. Whole mounts of organs were used.
(A) Eight-cell-stage embryo of line AtEM201. Bar = 20 μm.
(B) Heart-stage embryo of line AtEM201. Bar = 25 μm.
(C) Five-day-old seedling of AtEM201. Magnification ×10.
(D) Lateral root of a 10-day-old AtEM201 seedling. Magnification ×70.
(E) Heart-stage embryo of line AtEM101. Bar = 20 μm.
(F) Primary root of an AtEM101 7-day-old seedling. Magnification ×70.
(G) Transverse section of a primary root tip of an AtEM101 seedling in the region of the lateral root cap. Magnification ×70.
(H) GUS-positive initiating lateral root in an AtEM101 seedling primary root. Magnification ×70.
(I) Primary root tip of an AtBFM117 plant showing *COL* expression. Magnification ×70.

studies revealed that the mutations are in different genes (Topping et al., 1997). A second mutant, originally called *golftee*, was demonstrated by complementation studies to be allelic to the *gnom/emb30* mutation (Meinke, 1985; Mayer et al., 1991, 1993; Shevell et al., 1994).

hydra seedlings showed intersibling variability of phenotype but invariably were dwarfed, with an extremely short and wide hypocotyl and either a severely reduced or missing root system when grown in soil under greenhouse conditions or in vitro (Topping et al., 1997). The homozygous mutant is seedling lethal. Globular *hydra* embryos lack the organized cellular arrangement within both the upper and lower tiers that characterizes wild-type globular embryos, and torpedo-stage and early cotyledonary–stage *hydra* embryos lack the bilateral symmetry that is observed in the wild type, with *hydra* embryos being broadly globular in structure with no embryonic root apparent (Figures 2A and 2E). Sectioning of cotyledonary-stage embryos confirmed abnormalities of cell shape and filing, with no clearly distinguishable root primordium, although the hypophysis was present on a correctly organized suspensor. The mature embryo undergoes little elongation along the apical–basal axis and does not curl in the seed. The young *hydra* seedling (0 to 7 days postgermination) most commonly has five dark green cotyledons at the region of the shoot apex rather than the usual single pair (Figures 2B and 2F). Seedlings heterozygous or homozygous for the promoter trap T-DNA exhibited no detectable GUS activity, suggesting that the integrated T-DNA was either truncated at the left border (the site of the *gus* gene) or had inserted in the wrong orientation for activation of the promoterless *gus* reporter.

emb30 seedlings exhibited a variety of phenotypes, ranging from conical to spherical structures, which is consistent with other observations of *emb30* alleles identified in ethyl methanesulfonate–mutagenized and T-DNA populations (Mayer et al., 1993; Shevell et al., 1994). As for the other alleles described, this mutant lacks both shoot and root meristems, as determined by both anatomical and functional criteria.

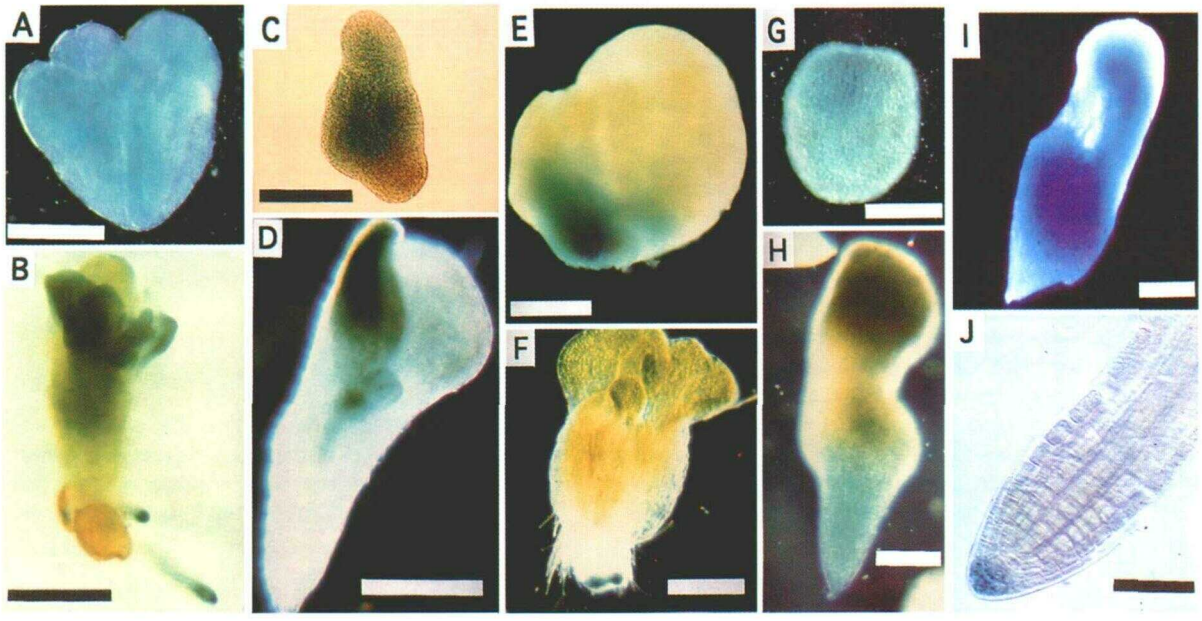

Figure 2. Expression of *EXO*, *PLS*, and *COL* Marker Genes in *emb30* and *hydra* Homozygous Mutant Backgrounds.

(A) to **(D)** show *EXO* expression, **(E)** to **(I)** show *PLS* expression, and **(J)** shows *COL* expression.
(A) *hydra* early cotyledonary–stage embryo. Bar = 25 μm.
(B) Seven-day-old *hydra* seedling. Bar = 500 μm.
(C) Cotyledonary-stage *emb30* embryo showing GUS activity in the upper region of the embryo only. Bar = 50 μm.
(D) Five-day-old *emb30* seedling. Bar = 200 μm.
(E) Late cotyledonary–stage *hydra* embryo. Bar = 25 μm.
(F) Five-day-old *hydra* seedling. Bar = 400 μm.
(G) *emb30* cotyledonary-stage embryo. Bar = 25 μm.
(H) Five-day-old *emb30* seedling with both morphological polarity and a polar distribution of *PLS* expression. Bar = 150 μm.
(I) Five-day-old *emb30* seedling with reduced morphological polarity and more diffuse *PLS* expression. Bar = 75 μm.
(J) Primary root of 10-day-old *hydra* seedling. Bar = 50 μm.

Marker Expression Patterns

Each of the three marker lines was crossed with plants heterozygous for the *hydra* and *emb30* mutations, respectively. F_2 progeny homozygous for each mutation and containing the respective *gus* markers were analyzed by histochemistry for the presence of GUS activity in torpedo-stage embryos and 7-day-old seedlings, and the results are presented in Figure 2. The *EXO* marker that is expressed in all wild-type embryonic cell types, except the suspensor, was also found to be constitutively expressed in *hydra* embryos (Figure 2A). In *hydra* seedlings, the pattern of GUS activity was essentially the same as that found in the wild type, that is, in the shoot and root apices (Figure 2B). In *emb30*, however, expression of *EXO* was more variable. In embryos and seedlings with the least severe mutant phenotype, expression was found to be restricted to the cotyledonary regions and was not observed in the root and shoot meristem (Figures 2C and 2D), although the more defective spherical embryos exhibited expression more uniformly. Structural polarity in *emb30* seedlings was readily recognized by the relatively high chlorophyll content of the upper cotyledonary region contrasting with the paler basal region, as described previously (Mayer et al., 1991, 1993). The *EXO* expression pattern is consistent with the interpretation that the *emb30* mutant allele studied has fused cotyledons but no functional or anatomically detectable shoot or root meristems.

The *PLS* marker, which is expressed in the basal region of the wild-type embryo, including the embryonic root primordium, was also found to be active in the basal region of the *hydra* embryos, although *hydra* has no morphologically obvious embryonic root (Figure 2E). Similarly, *PLS* is also active in the basal region of the *hydra* seedling, in the absence of correct root organogenesis (Figure 2F). This unexpected observation was also made for *emb30* mutants carrying this marker. Thus, *PLS* was also found to be expressed in *emb30* embryos (Figure 2G), even though the mutants have no root primordium (Mayer et al., 1993). Expression in *emb30* embryos was found to be spatially variable, typically in a less restricted pattern than is found in the wild type, although activity was often higher in the basal half of the *emb30* embryo. In AtEM101 seedlings, *PLS* was expressed in the primary root tip and developing and mature lateral root. Significantly, *PLS* is also expressed in the *emb30* seedlings (Figures 2H and 2I), although no root meristem was evident, as determined by either anatomical or functional criteria. *PLS* expression was found even in seedlings exhibiting the least obvious morphological polarity or organization (Figure 2I). The staining intensity and precise site of GUS activity showed some variability between *emb30* seedling siblings, with the most variability being in seedlings that showed the least evidence of morphological polarity (compare expression patterns in Figures 2H and 2I).

The *COL* marker was found to be active in the least abnormal *hydra* root tips (Figure 2J), supporting the anatomical observation that a root cap is present in such roots. *COL* was not expressed in any of the more than 300 *emb30* seedlings studied.

These observations suggest (1) that the markers *EXO* and *COL* on the one hand and *PLS* on the other resolve different components of the pathway of root development and (2) that *emb30* embryos and seedlings, which have been considered as putative root deletion mutants (Mayer et al., 1991, 1993), express at least one marker that is associated with embryonic and postembryonic root development in the wild-type plant. One interpretation of the pattern of *PLS* expression is that it is regulated in a way that is independent of anatomical or structural facets of root cell differentiation per se but rather reflects a biochemical differentiation of those cells in a position-related manner. This suggests that *PLS* may be activated by a signaling pathway that regulates position-dependent gene expression in the embryonic and seedling root.

Expression of *PLS* Is Induced Ectopically by Exogenous Auxin and Suppressed by Exogenous Cytokinin

To gain information on the processes that activate the *PLS* marker and that by implication might also determine positional information in polar organization, we conducted a set of experiments to determine whether auxins and cytokinins play a role in regulating *PLS* expression. Auxin has been implicated in the regulation of root development, both in vivo and in vitro (e.g., Schiavone and Cooke, 1987; Hinchee and Rost, 1992; Ferreira et al., 1994; Boerjan et al., 1995; Williams and Sussex, 1995). In a preliminary experiment to determine whether the expression of *PLS* was influenced by exogenous hormones, roots were excised from 8-day-old seedlings of the transgenic line AtEM101 and cultured for 5 days on a high-auxin medium containing 2.5 μM 2,4-D plus 0.25 μM kinetin. Histochemical staining for GUS activity in the cultured roots revealed intense ectopic expression in the treated tissues, particularly in callus tissue (Figure 3A). This response was not observed in the same medium lacking auxin, even though some callus formed. These results show that *PLS* expression can be induced in cells other than organized root tip cells in the presence of exogenous auxin.

To investigate quantitatively the effects of exogenous hormones on *PLS* expression in intact seedlings, we germinated seeds of transgenic line AtEM101, homozygous for the *PLS* gene fusion, on medium containing 0, 0.25, 2.5, 5, or 10 μM naphthalene acetic acid (NAA) or 0, 0.25, 2.5, 5, or 10 μM kinetin. Seedlings were harvested at intervals, examined phenotypically, and assayed for GUS activity. *PLS* expression increased when increased concentrations of auxin were applied. GUS activity in extracts of total seedlings grown from germination for 6 days in the presence of 10 μM NAA was approximately fourfold higher (mean activity of 2.07 ±SE at 0.24 nmol of 4-methylumbelliferone [MU] per mg of protein per min; $n = 10$) than in seedlings grown in the absence of auxin (mean activity of 0.61 ±SE at 0.15 nmol of

MU per mg of protein per min; $n = 10$; Figure 4A). A similar proportional increase in GUS activity was also found when 6-day-old AtEM101 seedlings were transferred to medium containing 10 μM NAA for 5 days, with steady state levels of the *PLS–gus* fusion transcript increasing dramatically within 5 hr of transfer (P. Chilley and K. Lindsey, unpublished data).

When 2.5 μM NAA was supplied to older seedlings (12 days postgermination) for 72 hr, there was an increase in the number of lateral roots formed, which is consistent with previous observations (Ferreira et al., 1994). For control seedlings transferred to fresh medium lacking NAA, the mean number of lateral roots was 130 ± 16 ($n = 20$) at day 15. For seedlings cultured for 12 days on medium lacking NAA and then for 3 days on 2.5 μM NAA, the mean number of lateral roots was 221 ± 12 ($n = 20$) at day 15, which marks an increase of 70% compared with the controls. One hundred percent of lateral root tips was found to exhibit *PLS* expression.

When seedlings were germinated and grown in the continuous presence of kinetin, the frequency of lateral root initiation was reduced compared with plants grown on cytokinin-free medium (data not shown). In contrast to the results for auxin-treated seedlings, however, the expression of *PLS* in the primary root tip was reduced, being barely detectable by histochemical analysis at the highest applied kinetin concentrations (data not shown). GUS activities were determined quantitatively to be ~30% of the activity (mean activity of 0.28 ±SE at 0.05 nmol of MU per mg of protein per min; $n = 10$) in seedlings treated for 6 days from germination with 10 μM kinetin compared with seedlings grown on hormone-free medium and approximatey sevenfold lower than seedlings grown on 10 μM NAA (Figure 4B).

To determine whether polar auxin transport is required for *PLS* expression, we germinated seedlings and grew them for 16 days on 0, 0.5, 1.0, 5.0, 7.5, or 10 μM triiodobenzoic acid (TIBA), an effective inhibitor of polar auxin transport (Liu et al., 1993) and examined them phenotypically and for GUS activity at intervals during development. TIBA inhibited lateral root formation in a dose-dependent way and also caused some reduction in the elongation growth of aerial parts (Figure 3B). No lateral roots were formed on seedlings grown for up to 16 days on 10 μM TIBA. Interestingly, *PLS* expression was still apparent in the primary root tip in the presence of 10 μM TIBA, although low levels of ectopic GUS activity were observed at the crown, presumably due to auxin accumulation. This result suggests that polar auxin transport is not required for *PLS* expression in the root tip.

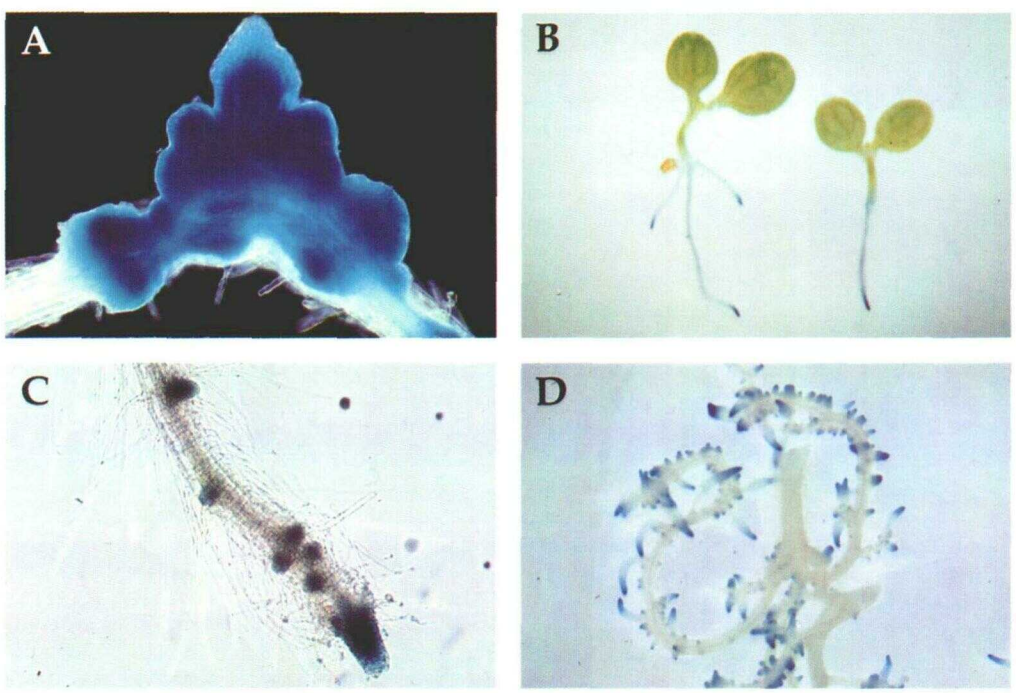

Figure 3. Effects of Exogenous Hormones, TIBA, and HU plus Auxin on AtEM101 Seedlings Stained to Reveal *PLS* Expression.

(A) Root of an AtEM101 seedling cultured for 5 days in the presence of 2.5 μM 2,4-D plus 0.25 μM kinetin. Magnification ×100.
(B) Six-day-old AtEM101 seedlings cultured in the absence (left) or presence (right) of 10 μM TIBA. Magnification ×8.
(C) and **(D)** Distal region of a primary root **(C)** and root system **(D)** of a 19-day-old seedling grown in the presence of 100 μM HU (2 days) followed by 100 μM HU plus 2.5 μM NAA for an additional 3 days. Magnification in **(C)** is 3100 and in **(D)** is 325.

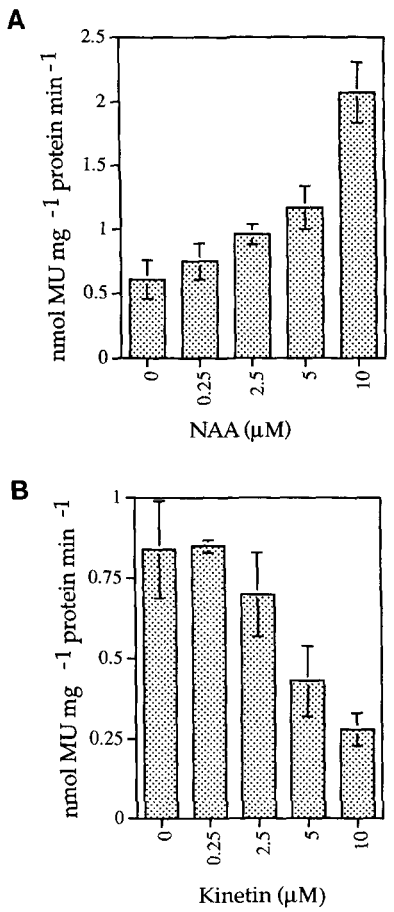

Figure 4. Effect of NAA Concentration and Kinetin Concentration on GUS Activity in AtEM101 Seedlings at 6 Days Postgermination.

Seeds were germinated in vitro in the presence of each concentration of either NAA or kinetin, and GUS activity was determined in total seedlings by fluorometric assay. Error bars indicate two standard errors of the means of 10 seedlings sampled per treatment.
(A) The effect of exogenous NAA concentration on GUS activity.
(B) The effect of exogenous kinetin concentration on GUS activity.

PLS Expression Is Independent of Extended Cell Division

In view of the observed activity of *PLS* in AtEM101 root tips, experiments were designed to determine whether *PLS* expression could be separated from cell division–related activities. *PLS* expression was therefore investigated in roots of seedlings grown in the presence of the cell division inhibitor hydroxyurea (HU). Furthermore, in an attempt to distinguish putative cell division–related inductive effects from auxin-related effects, seedlings were also treated with exogenous auxin either in the presence or absence of HU. In each case, seedlings were analyzed both for *PLS* expression and for effects on root development.

Effects of HU Treatment Alone

To inhibit cell division in the pericycle and primary root tip, we transferred 14-day-old seedlings of transgenic line AtEM101, homozygous for the *PLS* gene fusion, from HU-free and hormone-free medium to medium containing 10 or 100 μM HU for 5 days before analysis. HU at these concentrations has been demonstrated to be a potent cell cycle inhibitor, blocking DNA polymerase activity and S phase progression, and an inhibitor of lateral root cell division, even in the presence of auxin under these conditions (Ferreira et al., 1994). The expectation was that post–S phase cells would go through one round of mitosis and arrest at the G_1-to-S boundary, along with other cells in early S phase. Control 14-day-old seedlings were transferred to medium containing no HU or no auxin for 5 days before analysis. The results of the effects on lateral root production are presented in Table 1.

When seedlings of AtEM101 were transferred to medium containing HU alone, the frequency of lateral roots formed per unit length of root was slightly lower than for seedlings untreated with HU (Table 1), which is consistent with the view that HU inhibited further lateral root formation in the treated seedlings. Interestingly, it was found that neither the pattern nor the level of *PLS* GUS fusion activity was significantly different in untreated control seedlings (1.88 ±SE at 0.62 nmol of MU per mg of protein per min) and in seedlings treated with HU for 5 days (1.63 ± 0.15 nmol of MU per mg of proten per min). In contrast, for AtEM201 (*EXO*) plants, GUS activity was reduced in root tips of seedlings treated with HU

Table 1. Effects of NAA and HU on the Numbers of Lateral Roots Formed per Unit of Root Length of AtEM101 Seedlings

Treatment[a]	Mean Number of Lateral Roots per cm (19 dpg)[b]
Control (no NAA, no HU)	10.1 ± 0.9
10 μM HU	7.2 ± 0.8
100 μM HU	5.1 ± 1.1
0.25 μM NAA	18.3 ± 2.0
2.5 μM NAA	33.3 ± 2.9
10 μM HU + 0.25 μM NAA	60.3 ± 5.4
10 μM HU + 2.5 μM NAA	101.0 ± 13.7
100 μM HU + 0.25 μM NAA	67.7 ± 4.8
100 μM HU + 2.5 μM NAA	118.5 ± 14.9

[a]Fourteen-day-old seedlings were treated with NAA (0.25 or 2.5 μM) and/or HU (10 or 100 μM) separately for 5 days or given a 2-day pretreatment of HU (10 or 100 μM) followed by an additional 3 days on medium containing both HU and auxin (0.25 or 2.5 μM NAA).
[b]The number of lateral roots was determined as the mean of the total number of established roots, emerging roots, and initiated root primordia formed on the distal-most 1 cm of roots >1 cm in length 19 days postgermination (dpg) (see Figures 3C and 3D). Standard errors of the means are given. $n = 10$.

for 5 days (1.84 ± 0.16 nmol of MU per mg of protein per min) compared with seedlings grown in the absence of HU (2.42 ± 0.22 nmol of MU per mg of protein per min).

Effect of HU plus NAA

To determine whether there might be any auxin-mediated effects on *PLS* expression after HU-mediated inhibition of cell division, we transferred 14-day-old AtEM101 seedlings homozygous for the *PLS* gene fusion from HU-free and hormone-free medium to medium containing 10 or 100 μM HU alone as a pretreatment for 2 days. The seedlings were then transferrred to HU (10 or 100 μM) plus NAA (0.25 or 2.5 μM) for an additional 3 days. Fourteen-day-old seedlings transferred to medium containing 0.25 or 2.5 μM NAA alone for 5 days before analysis were used as controls.

The roots of seedlings treated with HU plus NAA showed a dramatic phenotype (Figures 3C and 3D). A significant increase in the number of sites of lateral root initiation was observed and detectable as foci of expanding cells. The ability of the cells at these sites to undergo extensive division was expected to be inhibited, however, as demonstrated by the effect of HU treatment alone (Table 1) and as shown previously (Ferreira et al., 1994). There was an ~10-fold increase in the numbers of rootlike structures formed in the presence of 100 μM HU plus 2.5 μM NAA as compared with untreated seedlings and a greater than threefold increase as compared with seedlings treated with 2.5 μM NAA alone (Table 1). The root phenotype after treatment was relatively short and "bushy," with numerous initiating primordia. Indeed, the determination of the numbers of primordia in seedlings treated with HU plus NAA is likely to be an underestimate because of the difficulty of counting in such dense root systems. The lateral roots formed contained variable numbers of cells, suggesting that each was derived from a different number of founder cells. Laskowski et al. (1995) estimate that Arabidopsis lateral roots originate from up to 11 founder cells in the pericycle. Similar qualitative results were also obtained for nontransgenic control seedlings and for other transgenic lines tested, indicating that this response was not specific to line AtEM101. Significantly, *PLS* expression in AtEM101 was detectable not only in the established primordia but also in the foci of expanded cells, further supporting the view that its activity is independent of extended cell division but is induced by auxin. Seedlings treated with the higher auxin concentration produced more lateral root initiation sites than did seedlings treated with the lower concentration, but essentially identical results were obtained with HU applied at either 10 or 100 μM (Table 1).

Control 14-day-old AtEM101 seedlings, transferred to medium supplemented with either 0.25 or 2.5 μM NAA in the absence of HU for 5 days, exhibited a dose-related increase in the number of lateral roots formed per unit of root length. Seedlings treated with 2.5 μM NAA for 5 days exhibited a threefold increase in the number of lateral roots per unit of root length compared with controls grown on NAA-free medium (Table 1). At this concentration of NAA, the length of roots formed was not significantly inhibited over a 5-day period, so the increase in lateral roots recorded after a treatment of HU plus NAA cannot be accounted for primarily by the same rate of root production in a "compressed" root system but rather by an increased rate of root initiation. This is consistent with our previous data (see above) showing an effect of NAA in increasing the total number of lateral roots per seedling. *PLS* expression was observed in all lateral roots after NAA treatment, as for untreated controls. However, expression in the root tips of auxin-treated seedlings was stronger than in those of the untreated controls (data not shown).

These results therefore show that auxin application to roots treated with HU does not result in *PLS* expression in cells other than in the correct position (i.e., in root tips), although the number of root tips formed increased significantly, presumably primarily through the expansion of cells that would be quiescent in roots not treated with HU.

DISCUSSION

It might be expected that pattern formation during plant embryogenesis is regulated by the nonuniform distribution of molecules that activate spatially restricted patterns of gene expression in the embryo. It is known, for example, that the establishment of bilateral symmetry in embryos of both *Brassica juncea* and Arabidopsis can be disrupted by the inhibition of polar auxin transport (Liu et al., 1993). In addition, the *SHOOT MERISTEMLESS* transcript, required for the establishment of the shoot apical meristem in Arabidopsis, is localized in a spatially restricted pattern as early as the globular stage of embryogenesis (Long et al., 1996), although the signals required to activate the gene have not been identified. Similarly, the Arabidopsis *ATLM1* gene, encoding a homeobox protein, is expressed in a polar fashion after the first asymmetric division of the zygote (Lu et al., 1996), and the gene encoding the Arabidopsis lipid transfer protein (*AtLTP1*) is expressed in a spatially restricted pattern and represents a marker of polarity, at least during later stages of embryogenesis (Vroemen et al., 1996). Other genes, such as *MONOPTEROS*, *GURKE* (Mayer et al., 1991; Berleth and Jürgens, 1993), *WUSCHEL* (Laux et al., 1996), and *HOBBIT* (Scheres et al., 1996), which have been defined genetically and found to be required for correct polar development, would be expected to be expressed in restricted patterns, but confirmation of this requires their cloning. Further progress in understanding the signaling events that define polarity during Arabidopsis embryogenesis requires the availability of new markers of apical–basal patterning.

PLS, *EXO*, and *COL* Mark Distinct Components of Root Development

We have analyzed the expression of three promoter trap *gus* fusion genes, which mark different components of axial development, in wild-type plants and in the *hydra* and *emb30* mutants. The use of *PLS*, *EXO*, and *COL* as markers provided interesting comparative information concerning the pathways of development in which the tagged genes may be involved and the organization of the shoot and root apical regions in *hydra* and *emb30* mutants.

Consistent with the fact that *emb30* fails to develop a root meristem during embryogenesis (Mayer et al., 1993) was the observation that this mutant exhibited no expression of *EXO* in the basal part of the torpedo-stage embryo that ultimately contributes to the seedling root (Scheres et al., 1994). However, *EXO* was expressed correctly in *hydra*, which failed to establish a morphologically recognizable embryonic root but could initiate an albeit morphologically abnormal seedling root and therefore has some embryonic root-associated meristematic activity (see Figure 2). In the meristemless *emb30*, however, the gene was expressed only in the cotyledonary region of embryos and seedlings and not in the relative positions at which seedling root or shoot meristems would be located. *EXO* would therefore appear to represent a marker not only of young cotyledonary tissues but also of shoot and root apical cells. Expression of this marker correlates with cell division activity. It is likely that some of the GUS activity in young cotyledons is due to stability of the GUS protein after *EXO* transcription in embryos.

PLS is expressed in a more spatially restricted pattern in embryos and seedlings of phenotypically wild-type plants than is *EXO*. *PLS* expression is found in the embryonic and seedling root and therefore does not represent a marker of generic apical–basal cell division activity in the way that *EXO* does. In support of this view is the observation that *PLS* expression can occur even after treatment with the cell division inhibitor HU. Such a treatment previously has been demonstrated to inhibit the expression of mitotic cyclin–*gus* gene fusions in lateral roots (Ferreira et al., 1994) and also reduces the level of *EXO* expression. This supports the views that HU treatment can suppress cell division and cell division–associated gene expression and that the GUS activity seen is not due principally to stability of the GUS protein synthesized before cell division inhibition. It is noteworthy that Foard et al. (1965) observed that lateral root initiation and polarized growth can occur in the absence of extended pericycle cell division in irradiated seedlings, which is consistent with the view that polar development does not require extended rounds of cell division activity.

Interestingly, and in contrast to *EXO*, *PLS* was expressed in the basal regions of *emb30* seedlings, which lack functional and anatomically recognizable roots (Figure 2). The implication of this observation, in combination with the cell division inhibition study, is that *PLS* is not a marker of the root meristem activity per se, because *emb30* lacks a root meristem; however, it does represent a marker of polarity or cell position. Certainly, transverse sections of roots of phenotypically wild-type plants expressing this marker show that it is expressed in a collection of cells at the root tip that have in common their position but not their lineage (Figure 1G). We can exclude the possibility that this GUS activity pattern is due to diffusion of the histochemical reaction intermediate, because the reaction was conducted in the presence of the oxidative catalyst potassium ferricyanide/ferrocyanide at concentrations that allow cell autonomous staining (Mascarenhas and Hamilton, 1992; Wei et al., 1997).

Our conclusion from these observations is that at least one root-associated gene expression pathway is active in cells of the basal region of embryos of *hydra* and seedlings of *emb30*, in the absence of correct morphological development or appropriate cell division activity. Although *PLS* expression was sometimes found most strongly in the basal region of *emb30* embryos, it was also found to be spatially variable in the mutant embryo, perhaps suggesting that morphologically variable *emb30* embryos are less able to compartmentalize gene expression patterns than are *emb30* seedlings. This is consistent with the variability of expression of the late embryogenesis polarity marker *AtLP1* in *emb30* mutants (Vroemen et al., 1996). We speculate that this may reflect an inability of this mutant to partition signaling molecules that regulate polarity. Because ectopic expression of *PLS* was induced by the exogenous application of the strong auxin 2,4-D and less strongly by treatment with the polar auxin inhibitor TIBA, it is possible that *emb30* mutants are unable to regulate correctly the distribution of endogenous auxin.

Currently, it is not clear how these data might relate to the observed cellular defects of the *emb30* mutant, which are in the regulation of cell shape (Mayer et al., 1993; Shevell et al., 1994). We can speculate that the aberrant *PLS* expression may be associated with the inability of the *emb30* mutant to establish correctly a shoot meristem, normally a source of auxin that is transported in a basipetal manner. Consistent with this is the observation that reduced polar auxin transport, produced by either chemical inhibitor treatment or mutation of the *pin* gene (Liu et al., 1993), causes an embryonic phenotype similar to that observed in some *emb30* seedlings. The fact that the *COL* marker was expressed in at least some postembryonic roots of *hydra* but not in *emb30* suggests that, like *EXO*, it is a marker of cell differentiation rather than position.

Establishment of Different Components of Root Development: A Model

We conclude that *HYDRA*, *EXO*, and *COL* mark pathways of cellular organization that are distinct from the establishment of polarity at a multicellular level, for which *PLS* is a marker (Figure 5). We also consider the *EMB30* gene to play an indirect role in regulating polarity, because the structural polarity and

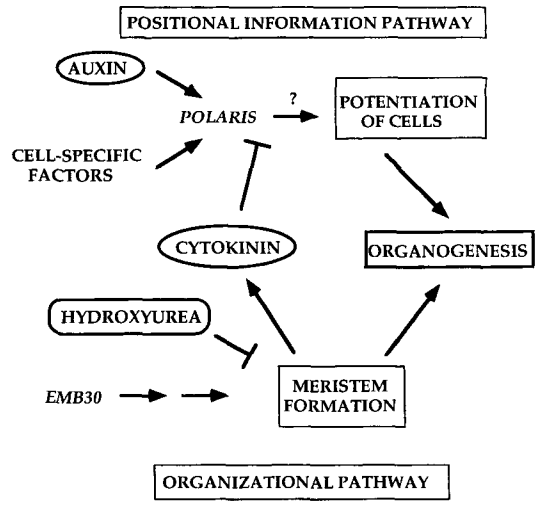

Figure 5. Model to Describe the Relationship of *PLS* Expression, *EMB30* Action, and Root Development in Arabidopsis.

EMB30 expression is required for root meristem formation, but *PLS* expression is independent of *EMB30* and root meristem development and activity, as evidenced by *PLS* expression in *emb30* mutants and after HU treatment. *PLS* may therefore represent a marker for a positional information pathway of polar organization, which is distinct from an *EMB30*-dependent pathway of meristem formation. *PLS* expression is activated by auxin and repressed by cytokinin. In this model, we make the assumption that the interactions between auxins, cytokinins, and *PLS* are similar both during embryogenesis and in seedling root development. When active seedling root meristems are inhibited by HU treatments in the presence of auxin, many pericycle cells attempt to form new lateral roots, suggesting that active meristems produce an inhibitor of new meristems; this inhibitor may be a cytokinin, which is antagonistic to the inductive effects of auxin both in regulating meristem formation or activity and in *PLS* expression. Because exogenous auxin does not induce *PLS* expression in all cell types, we speculate that other cell-specific factors must interact with auxin to regulate its expression. This is consistent with the view that auxin, in combination with these factors, plays a role in defining positional information in the developing Arabidopsis seedling.

pattern of *PLS* expression in a single *emb30* mutant allele are variable between siblings, although each sibling carries the same *emb30* mutation. The implication of this is that EMB30 is not a regulator of patterning per se, but its effect on cell morphogenesis influences polar organization of the multicellular embryo and seedling. We speculate that this may be the consequence of a failure in the regulation of auxin distribution in the *emb30* embryo or seedling that is linked to the level of structural disorganization. Clearly, EMB30 is not required for transcriptional activation of *PLS*; therefore, each can be considered to also mark different regulatory pathways. We propose that both the positional information pathway, marked by the *PLS* gene, and the organizational pathway are required for correct root organogenesis, as illustrated in Figure 5.

We can extend this model to support the view that long-range signals, such as auxins and cytokinins, may play a role in activating or suppressing root meristem activity. We found that auxin potentiated pericycle cells for fates as meristem cells, coordinate with an activation of *PLS* expression, independently of cell division. As indicated in Figure 5, auxin, cytokinin, and other cell-specific factors regulate *PLS* expression, but the role of the *PLS* gene itself is currently unknown. We suggest that cytokinin, originating from newly developing meristems, may regulate the position of other meristems by functioning as an inhibitor of their formation nearby. This could account for the increased frequency of lateral root formation in seedlings treated with HU plus NAA, in which meristem activity (and so cytokinin production) would be inhibited (Figure 5). Thus, the spacing of lateral roots might be achieved through an antagonistic interaction between auxin and cytokinin. This is not a new concept (e.g., McCully, 1975). The *alf3* mutant, which is characterized by the formation of new lateral root meristems very close to dead or dying root tips, provides additional evidence (Celenza et al., 1995). Interestingly, the *alf3* phenotype can be rescued by the application of indole, a precursor of auxin. The ability to separate cell division processes (whether by mutation or by chemical inhibition) from the expression of root tip markers, such as *PLS*, provides a new route to readdress the nature of positional signaling in roots. One interesting point to come from the NAA/HU experiments is that a large number of pericycle cells are competent to redifferentiate into lateral root cells (as marked by their de novo expression of *PLS*) and are presumably repressed from this change in fate in response to local inhibitory signals. The HU-mediated inhibition of meristem activity alone is not enough to activate these cells; exogenous auxin is also required. Therefore, we propose that the observed greater effect of auxin on lateral root formation after HU treatment is a consequence of the inhibitory effect of HU on meristems to divide and hence to produce inhibitors, perhaps cytokinin (see Figure 5).

In further support of this concept, it has been demonstrated that auxin accumulates in Arabidopsis root tips and may regulate root cell division activity (Kerk and Feldman, 1995). It is known that high auxin-to-cytokinin ratios can induce primary and lateral root formation in tissue and root cultures of Arabidopsis and a number of other species (e.g., Valvekens et al., 1988; Ferreira et al., 1994; Williams and Sussex, 1995) and that inhibitors of auxin transport or action can interfere with the processes of root morphogenesis (Schiavone and Cooke, 1987; Hinchee and Rost, 1992; Fischer and Neuhaus, 1996). In addition, the *superroot* (Boerjan et al., 1995), *rooty* (King et al., 1995), and *alf1* mutants (Celenza et al., 1995) of Arabidopsis, which produce abnormally high levels of auxin, and transgenic overproducers of auxin (Kares et al., 1990) also produce excessive numbers of lateral roots. The auxin-insensitive mutants of

Arabidopsis, however, are defective in lateral root formation (Hobbie and Estelle, 1995). As indicated in Figure 5, other local, cell-specific signals or receptors must be required to mediate any inductive effects of auxin, because exogenous auxin does not strongly induce *PLS* expression or root development in all cell types in the seedling.

Apparently inconsistent with the possibility that auxin is a regulator of the root positioning pathway marked by *PLS*, TIBA did not inhibit *PLS* expression in the primary root tip, although exogenous cytokinin did. Because the primary root is established in the embryo rather than during postembryonic processes and *PLS* is expressed in both embryo and seedling, one interpretation is that the signals required to maintain root meristem identity in seedlings are established in the embryo and are retained in postgerminative processes. However, these do not appear to be dependent on polar auxin transport in either seedlings or embryos, because inhibitor treatment of cultured *B. juncea* embryos (pre-heart stage) and polar transport–defective *pin* mutants of Arabidopsis are able to develop embryonic roots (Liu et al., 1993). However, this is in contrast to the observation that TIBA treatment can inhibit root meristem development in the wheat embryo (Fischer and Neuhaus, 1996), and the discrepancy here may be due to the extent by which polar auxin transport was inhibited. The possibility that the embryonic and seedling root apices of Arabidopsis synthesize auxin, which might explain *PLS* expression in that position, cannot be excluded at present. In support of this possibility, Feldman (1981) and Evans (1984) describe the production of auxin by root tips in other species. The coordinate expression of *PLS*, *EXO*, and *COL* in both primary and lateral roots suggests that at least some components of the signaling systems that maintain root tip gene expression patterns are common to both. This view is further strengthened by observations of overlapping enhancer trap expression patterns in embryonic and lateral roots of Arabidopsis (Malamy and Benfey, 1997).

The results presented in this study are consistent with a general view currently emerging that pathways of cytodifferentiation, morphogenesis, and pattern formation in Arabidopsis embryogenesis are regulated independently. Thus, Torres-Ruiz and Jürgens (1994) argued that the *fass* mutant of Arabidopsis maintains pattern formation in the absence of correct cell morphogenesis; the *raspberry* mutant shows evidence of embryonic cell differentiation in the absence of morphogenesis (Yadegari et al., 1994). Furthermore, analysis of the *tangled-1* mutant of maize shows that polar growth in plants does not require correct cell division orientation (Smith et al., 1996). Both the *EMB30* and *HYDRA* genes are required for the generation of correct cell shape, and Shevell et al. (1994) discussed in some detail the possibility that the *emb30* mutant phenotype is a consequence of incorrect secretory processes such that proper control over cell differentiation and/or cell wall positioning is lacking. The expression of *PLS* in these mutants further shows a separation of morphogenesis and pattern formation. Future work will be directed toward understanding more of the nature of the signals that activate the *PLS* and *EXO* genes and determining the functions of the gene products.

METHODS

Plant Material

Transgenic plants (*Arabidopsis thaliana* ecotype C24) were generated by *Agrobacterium tumefaciens*–mediated infection and regeneration of root tissue explants, as described previously (Clarke et al., 1992). Plants contained the promoter trap binary vector pΔgusBin19, which contains a promoterless β-glucuronidase (*gus*) gene and a constitutively expressed neomycin phosphotransferase II (*nptII*) gene conferring kanamycin resistance to transformants (Topping et al., 1991). Seeds were bulked from independent transgenic lines, and plants were grown in the greenhouse as described previously (Topping et al., 1994).

Populations of mixed T_3 seed were screened for seedling-defective mutants in vitro. Seeds were surface sterilized (Clarke et al., 1992) and plated on half-strength Murashige and Skoog medium (Sigma), 1% sucrose, and 0.8% agar (Difco, Detroit, MI) at 22 ± 2°C and at a photon flux density of ~150 μmol m^{-2} sec^{-1}. Seeds were vernalized at 4°C on half-strength Murashige and Skoog medium for 2 days in the dark. GUS marker lines were identified as described previously (Lindsey et al., 1993; Topping et al., 1994).

Because both *emb30* and *hydra* mutants are seedling lethal, genetic crosses for *emb30/golftee* complementation studies and for introducing GUS markers into both *hydra* and *emb30* backgrounds were achieved by crossing plants heterozygous for the respective mutations and screening F_2 progeny.

Tissue Culture

The effect of auxin on *POLARIS* (*PLS*) expression in roots was performed by culturing roots aseptically at 25°C in continuous light on a medium containing relatively high auxin concentrations (Gamborg's B5 medium [Sigma]; Gamborg et al., 1968) containing 0.5 mg/L 2,4-D, 0.05 mg/L kinetin, 0.5 g/L Mes, and 20 g/L glucose, pH 5.8, solidified with 8 g/L Difco Bacto agar. Roots were excised from 8-day-old seedlings and cultured for 5 days before histochemical staining for GUS activity.

GUS Analysis

A quantitative GUS assay was conducted fluorometrically with crude protein extracts, according to Jefferson et al. (1987), by using a fluorimeter (model RF5001PG; Shimadzu Europa, Milton Keynes, UK). Tissue localization of GUS enzyme activity was determined by staining for up to 12 hr at 37°C in 1 mM 5-bromo-4-chloro-3-indolyl β-D-glucuronic acid (X-gluc; Melford Laboratories, Suffolk, UK), essentially according to the method of Jefferson et al. (1987). The protocol was modified by the use of buffer comprising 100 mM sodium phosphate, pH 7.0, 10 mM EDTA, 0.1% (v/v) Triton X-100 (A.-M. Stomp, editorial in U.S. Biochemical's publication Editorial Comments, Vol. 16, No. 4 [1990]), and 1 mM each of potassium ferricyanide and potassium ferrocyanide to inhibit diffusion of the reaction intermediate. Stained tissues were cleared of chlorophyll by soaking in 70% (v/v)

ethanol. For the preparation of histological sections, plant tissues stained with X-gluc were fixed and embedded in Historesin (Jung/Leica, Heidelberg, Germany), essentially according to the manufacturer's instructions. Tissue was vacuum infiltrated with the fixative 4% (w/v) paraformaldehyde, dehydrated in an ethanol series, and embedded in Historesin. Sections 10-μm thick were cut on a Bright rotary retracting microtome (Bright Instrument Co. Ltd., Huntingdon, UK). Photographs were taken on Ektachrome 160 tungsten-balanced film, using Nikon Optiphot (Nikon UK Ltd., Kingston, UK), Zeiss Axioskop, and Zeiss Stemi SV8 (Carl Zeiss Ltd., Welwyn Garden City, UK) microscopes.

Hormone and Inhibitor Application Experiments

To investigate the effects of exogenous hormones and triiodobenzoic acid (TIBA; Sigma) on *PLS* expression, seeds of AtEM101 homozygous for the *PLS* gene fusion were germinated aseptically, as described above, on half-strength Murashige and Skoog medium containing up to 10 μM 1-naphthaleneacetic acid (NAA), kinetin, or TIBA, according to the particular experiment, and assayed for GUS activity at 6 days postgermination.

To investigate the effect of hydroxyurea (HU; Sigma), either alone or in the presence of auxin, on *PLS* expression, 14-day-old AtEM101 seedlings homozygous for the *PLS* gene fusion were transferred from HU-free and hormone-free medium to medium containing 10 or 100 μM HU, either with or without 0.25 or 2.5 μM NAA, for 5 days before analysis. To inhibit cell division in the pericycle, HU was supplied alone, as a pretreatment for 2 days, before seedlings were transferred to HU (10 or 100 μM) plus NAA (0.25 or 2.5 μM) for an additional 3 days. For controls, 14-day-old seedlings were transferred (1) to medium containing no HU or no auxin for 5 days before analysis, (2) to medium containing 0.25 or 2.5 μM NAA alone before analysis, and (3) to medium containing either 10 or 100 μM HU alone before analysis. Mean GUS activities were determined for five replicate seedlings or root systems per treatment, and the standard errors of the means are presented. The frequency of lateral root formation was determined as the mean total number of established roots, emerging roots, and initiated root primordia formed on the distal-most 1 cm of roots >1 cm in length 19 days postgermination.

ACKNOWLEDGMENTS

We acknowledge the Biotechnology and Biological Sciences Research Council and Commission of the European Communities BRIDGE program for financial support of this work. We are also grateful to Drs. Diane Shevell and Nam-Hai Chua for supplying *emb30* seed for complementation analysis, Vanessa May for assistance with growing plants and GUS histochemistry, and Geke Mollema for assistance with tissue culture work.

Received April 10, 1997; accepted August 12, 1997.

REFERENCES

Berleth, T., and Jürgens, G. (1993). The role of the *monopteros* gene in organising the basal body region of the *Arabidopsis* embryo. Development **118,** 575–587.

Boerjan, W., Cervera, M.-T., Delarue, M., Beeckman, T., Dewitte, W., Bellini, C., Caboche, M., Van Onckelen, H., Van Montagu, M., and Inzé, D. (1995). *superroot*, a recessive mutation in Arabidopsis, confers auxin overproduction. Plant Cell **7,** 1405–1419.

Celenza, J.L., Grisafi, P.L., and Fink, G.R. (1995). A pathway for lateral root formation in *Arabidopsis thaliana*. Genes Dev. **9,** 2131–2142.

Clarke, M.C., Wei, W., and Lindsey, K. (1992). High frequency transformation of *Arabidopsis thaliana* by *Agrobacterium tumefaciens*. Plant Mol. Biol. Rep. **10,** 178–189.

Coen, E.S., and Meyerowitz, E.M. (1991). The war of the whorls: Genetic interactions controlling flower development. Nature **353,** 31–37.

Coen, E.S., Nugent, J.M., Luo, D., Bradley, D., Cubas, P., Chadwick, M., Copsey, L., and Carpenter, R. (1995). Evolution of floral symmetry. Philos. Trans. R. Soc. Lond. B **350,** 35–38.

Dolan, L., Janmaat, K., Willemsen, V., Linstead, P., Poethig, S., Roberts, K., and Scheres, B. (1993). Cellular organisation of the *Arabidopsis thaliana* root. Development **119,** 71–84.

Eady, C., Lindsey, K., and Twell, D. (1995). The significance of microspore division and division symmetry for vegetative cell-specific transcription and generative cell differentiation. Plant Cell **7,** 65–74.

Evans, M.L. (1984). Functions of hormones at the cellular level of organization. In Hormonal Regulation of Development, Vol. 2, T.K. Scott, ed (Heidelberg, Germany: Springer-Verlag), pp. 23–79.

Feldman, L.J. (1981). Effect of auxin on acropetal auxin transport in roots of corn. Plant Physiol. **67,** 278–281.

Ferreira, P.C.G., Hemerley, A.S., de Almeida Engler, J., Van Montagu, M., Engler, G., and Inzé, D. (1994). Developmental expression of the Arabidopsis cyclin gene *cyc1At*. Plant Cell **6,** 1763–1774.

Fischer, C., and Neuhaus, G. (1996). Influence of auxin on the establishment of bilateral symmetry in monocots. Plant J. **9,** 659–669.

Foard, D.E., Haber, A.H., and Fishman, T.N. (1965). Initiation of lateral root primordia without completion of mitosis and without cytokinesis in uniseriate pericycle. Am. J. Bot. **52,** 580–590.

Gamborg, O.L., Miller, R.A., and Ojima, K. (1968). Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell Res. **50,** 151–158.

González-Reyes, A., Elliott, H., and St. Johnston, D. (1995). Polarization of both major body axes in Drosophila by *gurken-torpedo* signaling. Nature **375,** 654–658.

Hinchee, M.A.W., and Rost, T.L. (1992). The control of lateral root development in cultured pea seedlings. II. Root fasciation induced by auxin inhibitors. Bot. Act. **105,** 121–126.

Hobbie, L., and Estelle, M. (1995). The *axr4* auxin-resistant mutants of *Arabidopsis thaliana* define a gene important for root gravitropism and lateral root initiation. Plant J. **7,** 211–220.

Jefferson, R.A., Kavanagh, T.A., and Bevan, M.W. (1987). GUS fusions: β-Glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO J. **6,** 3901–3907.

Kares, C., Prinsen, E., Van Onckelen, H., and Otten, L. (1990). IAA synthesis and root induction with *iaa* genes under heat shock promoter control. Plant Mol. Biol. **15,** 225–236.

Kerk, N., and Feldman, L.J. (1995). A biochemical model for the initiation and maintenance of the quiescent centre: Implications for organisation of root meristems. Development **121,** 2825–2833.

King, J.J., Stimart, D.P., Fisher, R.H., and Bleecker, A.B. (1995). A mutation altering auxin homeostasis and plant morphology in Arabidopsis. Plant Cell **7,** 2023–2037.

Kornberg, T.B., and Tabata, T. (1993). Segmentation of the Drosophila embryo. Curr. Opin. Genet. Dev. **3,** 585–593.

Laskowski, M.J., Williams, M.E., Nusbaum, H.C., and Sussex, I.M. (1995). Formation of lateral root meristems is a two-stage process. Development **121,** 3303–3310.

Laux, T., Mayer, K.F.X., Berger, J., and Jürgens, G. (1996). The *WUSCHEL* gene is required for shoot and floral meristem integrity in Arabidopsis. Development **122,** 87–96.

Lindsey, K., Wei, W., Clarke, M.C., McArdle, M.F., Rooke, L.M., and Topping, J.F. (1993). Tagging genomic sequences that direct transgene expression by activation of a promoter trap in plants. Transgenic Res. **2,** 33–47.

Lindsey, K., Topping, J.F., da Rocha, P.S.C.F., Horne, K.L., Muskett, P.R., May, V.J., and Wei, W. (1996). Insertional mutagenesis to dissect embryonic development in Arabidopsis. In Embryogenesis: Generation of a Plant, T. Wang and A.C. Cuming, eds (Oxford, UK: Bios), pp. 51–76.

Liu, C.-m., Xu, Z.-h., and Chua, N.-H. (1993). Auxin polar transport is essential for the establishment of bilateral symmetry during early plant embryogenesis. Plant Cell **5,** 621–630.

Long, J.A., Moan, E.I., Medford, J.I., and Barton, M.K. (1996). A member of the KNOTTED class of homeodomain proteins encoded by the *STM* gene of Arabidopsis. Nature **379,** 66–69.

Lu, P., Porat, R., Nadeau, J.A., and O'Neill, S.D. (1996). Identification of a meristem L1 layer–specific gene in Arabidopsis that is expressed during embryonic pattern formation and defines a new class of homeobox genes. Plant Cell **8,** 2155–2168.

Malamy, J.E., and Benfey, P.N. (1997). Organization and cell differentiation in lateral roots of *Arabidopsis thaliana*. Development **124,** 33–44.

Mansfield, S.G., and Briarty, L.G. (1991). Early embryogenesis in *Arabidopsis thaliana*. II. The developing embryo. Can. J. Bot. **69,** 461–476.

Mascarenhas, J.P., and Hamilton, D.A. (1992). Artifacts in the localization of GUS activity in anthers of petunia transformed with a CaMV 35S–GUS construct. Plant J. **2,** 405–408.

Mayer, U., Torres Ruiz, R.A., Berleth, T., Misera, S., and Jürgens, G. (1991). Mutations affecting body organization in the Arabidopsis embryo. Nature **353,** 402–407.

Mayer, U., Büttner, G., and Jürgens, G. (1993). Apical–basal pattern formation in the Arabidopsis embryo: Studies on the role of the *gnom* gene. Development **117,** 149–162.

McCully, M.E. (1975). The development of lateral roots. In The Development and Function of Roots, J.G. Torrey and D.T. Clarkson, eds (London: Academic Press), pp. 105–124.

Meinke, D.W. (1985). Embryo-lethal mutants of *Arabidopsis thaliana*: Analysis of mutants with a wide range of lethal phases. Theor. Appl. Genet. **69,** 543–552.

Quatrano, R.S., Brian, L., Aldridge, J., and Schultz, T. (1991). Polar axis fixation in Fucus zygotes: Components of the cytoskeleton and extracellular matrix. Development **1** (suppl.), 11–16.

Scheres, B., Wolkenfelt, H., Willemsen, V., Terlouw, M., Lawson, E., Dean, C., and Weisbeek, P. (1994). Embryonic origin of the *Arabidopsis* primary root and root meristem initials. Development **120,** 2475–2487.

Scheres, B., McKhann, H.I., and Van den Berg, C. (1996). Roots redefined: Anatomical and genetic analysis of root development. Plant Physiol. **111,** 959–964.

Schiavone, M., and Cooke, T. (1987). Unusual patterns of embryogenesis in the domesticated carrot: Developmental effects of exogenous auxins and auxin transport inhibitors. Cell Differ. **21,** 53–62.

Shevell, D.E., Leu, W.-M., Gillmour, C.S., Xia, G., Feldmann, K.A., and Chua, N.-H. (1994). *EMB30* is essential for normal cell division, cell expansion, and cell adhesion in Arabidopsis and encodes a protein that has similarity to Sec7. Cell **77,** 1051–1062.

Smith, L.G., Hake, S., and Sylvester, A.W. (1996). The *tangled-1* mutation alters cell division orientations throughout maize leaf development without altering leaf shape. Development **122,** 481–489.

Terasaka, O., and Niitsu, T. (1987). Unequal cell division and chromatin differentiation in pollen grain cells. I. Centrifugal, cold and caffeine treatments. Bot. Mag. Tokyo **100,** 205–216.

Topping, J.F., Wei, W., and Lindsey, K. (1991). Functional tagging of regulatory elements in the plant genome. Development **112,** 1009–1019.

Topping, J.F., Agyeman, F., Henricot, B., and Lindsey, K. (1994). Identification of molecular markers of embryogenesis in *Arabidopsis thaliana* by promoter trapping. Plant J. **5,** 895–903.

Topping, J.F., May, V.J., Muskett, P.R., and Lindsey, K. (1997). Mutations in the *HYDRA1* gene of *Arabidopsis* perturb cell shape and disrupt embryonic and seedling morphogenesis. Development, in press.

Torres-Ruiz, R.A., and Jürgens, G. (1994). Mutations in the *FASS* gene uncouple pattern formation and morphogenesis in *Arabidopsis* development. Development **120,** 2967–2978.

Valvekens, D., Van Montagu, M., and Van Lijsebettens, M. (1988). *Agrobacterium tumefaciens*–mediated transformation of *Arabidopsis thaliana* root explants by using kanamycin selection. Proc. Natl. Acad. Sci. USA **85,** 5536–5540.

Vroemen, C.W., Langeveld, S., Mayer, U., Ripper, G., Jürgens, G., Van Kammen, A., and De Vries, S. (1996). Pattern formation in the Arabidopsis embryo revealed by position-specific lipid transfer protein gene expression. Plant Cell **8,** 783–791.

Wei, W., Twell, D., and Lindsey, K. (1997). A novel nucleic acid helicase gene identified by promoter trapping in *Arabidopsis*. Plant J. **11,** 1307–1314.

Williams, M.E., and Sussex, I.M. (1995). Developmental regulation of ribosomal protein L16 genes in *Arabidopsis thaliana*. Plant J. **8,** 65–76.

Yadegari, R., de Paiva, G.R., Laux, T., Koltunow, A.M., Apuya, N., Zimmerman, J.L., Fischer, R.L., Harada, J.J., and Goldberg, R.B. (1994). Cell differentiation and morphogenesis are uncoupled in Arabidopsis *raspberry* embryos. Plant Cell **6,** 1713–1729.