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Embryonic origin of the *Arabidopsis* primary root and root meristem initials

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SUMMARY

The embryonic origin of the *Arabidopsis* root and hypocotyl region has been investigated using histological techniques and clonal analysis. Our data reveal the pattern of cell division in the embryo giving rise to the various initials within the root promeristem. A small region of the root at its connection with the hypocotyl appears not to be derived from the promeristem initials. This region contains two cortical cell layer and [^{3}H]thymidine incorporation data suggest that it lacks postembryonic cell divisions. Sectors marked by transposon excision from the β-glucuronidase marker gene are used to investigate cell lineages giving rise to root and hypocotyl. The position of end points from sectors with embryonic origin show little variation and hence reveal preferred positions in the seedling for cells derived from different regions of the embryo. The radial extent of complete root sectors is consistent with the radial arrangement of root meristem initials at the heart stage of embryogenesis inferred from histological analysis. Using the clonal data, a fate map is constructed depicting the destiny of heart stage embryonic cell tiers, in the seedling root and hypocotyl. The variability in the sector end points indicates that distinct cell lineages are not restricted for root or hypocotyl fate. In contrast, derivatives of the hypophyseal cell do appear to be restricted to the columella and central cell region of the root.

Key words: *Arabidopsis*, root, meristem, embryogenesis, clonal analysis, *Ac* transposon

INTRODUCTION

In most angiosperm plant species groups of cells representing the two primary meristems are laid down during embryogenesis (Natesh and Rau, 1984). Upon germination the primary root and shoot meristems initiate postembryonic development. The meristematic origin of differentiated cells in the shoot generally cannot be deduced from histological data. In roots, on the other hand, a simple relationship between differentiated cells and meristematic cells can be presumed. Cells within root meristems are located at the distal end of continuous cell files extending into the mature root. From histological analysis it has been concluded that cell files in roots from some species converge towards a single initial, while in other species separate tiers of initials for different cell types ('meristem layers') appear to exist (Steeves and Sussex, 1989). Histological studies on the embryonic origin of root meristem initials in crucifers have been performed in *Brassica napus* (Tykarska, 1979; Kuras, 1980) and *Arabidopsis thaliana* (Dolan et al., 1993). These studies indicate that formation of a lateral root cap layer can be viewed as hallmarking the onset of root meristem activity at the heart stage of embryogenesis. Using this formative division as a marker, a characteristic division pattern of a set of initials surrounding a small quiescent centre, schematised in Fig. 1, has been proposed to occur from the heart stage onward. A minimal set of initials, as a construction centre for the root, has been coined the 'promeristem' (Clowes, 1954). It has been inferred that this characteristic pattern of divisions within the promeristem is perpetuated after germination, giving rise to the seedling root structure (Dolan et al., 1993).

The proposed presence of a root promeristem at the heart stage of embryogenesis by no means settles all questions regarding the ontogeny of the seedling root. The first question in need of further exploration is whether the relationship between initial cells and their derivatives, as predicted from histological analysis (cf. Fig. 1), is supported by an independent lineage analysis. A second question is whether characteristic embryonic cell divisions separate root and hypocotyl regions, which has a bearing on the more general issue of whether developmental compartments are set apart during plant embryogenesis. Regular divisions in the *Arabidopsis* embryo could be interpreted as a reflection of the creation of 'developmental compartment boundaries' (Garcia-Bellido et al., 1976) with fixed, clonally propagated fates. Alternatively, positional information may be superimposed on boundaries created by cell division. Evidence for the latter viewpoint comes both from histological observations on different species often with irregular embryo division patterns (cf Natesh and Rau, 1984), and from clonal analysis of embryogenesis (e.g. Poethig et al., 1986).

Further insight into the questions posed above relies on

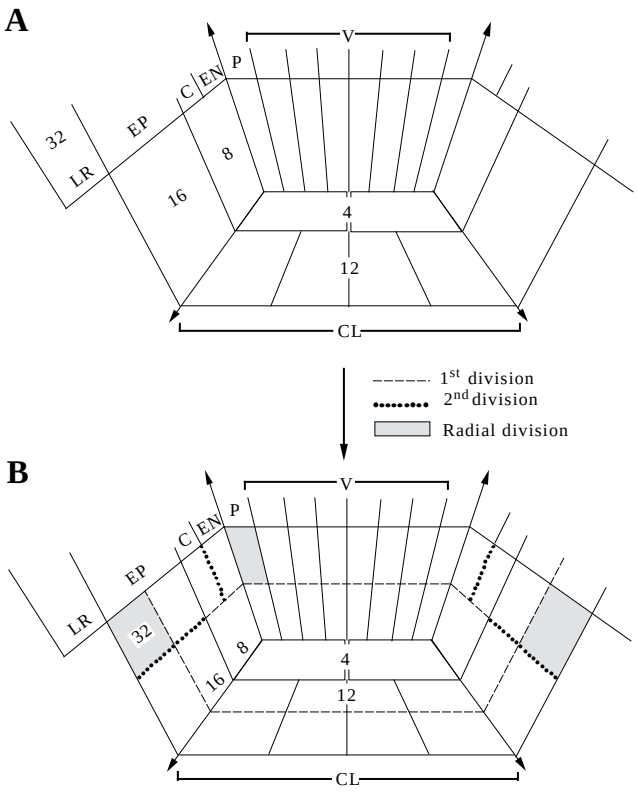


Fig. 1. The *Arabidopsis* root promeristem. (A) Location and numbers of initial cells for all tissues surrounding 4 central quiescent cells. (B) Division patterns in the promeristem: formation of new initials and tissue types. Division planes as indicated in the legend. LR, lateral root cap; EP, epidermis; C, cortex; EN, endodermis; P, pericycle; CL, columella.

unequivocal establishment of clonal relationships. Clonal analysis of plant development is based on the size and distribution of genetically marked somatic sectors in seedlings or adult plants. In studies on clonal relations within shoots, such sectors have been determined mainly by acute irradiation at a defined developmental stage to induce or uncover easily scorable genetic markers in a single cell (reviewed by Poethig, 1987). The vertical extent and width of resulting sectors are combined with histological analysis at the time of irradiation. This allows reconstruction of the probable participation of particular shoot meristem cells in the development of a given organ (Poethig et al., 1986; Jegla and Sussex, 1989; Irish and Sussex, 1992; Furner and Pumfrey, 1992, 1993). These studies revealed a lack of correlation between cells in the shoot meristem and ultimate cell differentiation fates. Late, lineage-independent acquisition of cell fate was also evident from analysis of sectors induced at various stages of leaf development (Poethig and Sussex, 1985).

Clonal analysis of primary roots has been performed in several species using acute irradiation of postembryonic root tips. Brumfield (1943) found chimeric roots of *Crepis capillaris* and *Vicia faba* to be triangular in cross section. From these data he inferred the presence of three initial cells in the root, each of which contributing to all tissues. Clowes (1959) showed that the use of X-rays may activate otherwise quiescent cells to replace damaged initials. Taking this replacement phenomenon into account, a larger number of initials was inferred from X-ray induced clones in *Vicia faba* (Davidson, 1961). In these and similar studies no firm link with the embryonic development of the root primordium was provided.

We present a lineage analysis of the cells from the hypocotyl, root and root meristem of *Arabidopsis thaliana*. Clonal analysis on the root and hypocotyl region of *Arabidopsis* seedlings can provide a framework for subsequent genetic dissection of root development. Excision of a maize *Ac* element from a β-glucuronidase (*GUS*) gene is used to mark cell clones. Expression of 35S-*GUS* fusions resulting from excision is easily monitored and the 35S promoter has the potential to be active in all root tissues (Benfey et al., 1990). Furthermore, excision out of the *GUS* transgene is not expected to perturb normal root development, unlike irradiation. Analysis of the end points of embryonic sectors revealed preferred positions in the seedling, corresponding to subdivisions of embryonic cell tiers. A strong, but not absolute, correlation was found between the subdivision of the basal tier of the triangular *embryo proper* and the separation of root and hypocotyl cell fates. Furthermore, the analysed sectors support the notion of a small set of initials, separated at the mid heart stage of embryogenesis, which form the root promeristem. These initials remain active during a period of postembryonic development. [^{3}H]thymidine incorporation data as well as sector analysis revealed that part of the root is not derived from these initials but from a separate tier of embryonic cells. Both the relatively fixed embryonic origin and the limited anatomical complexity of the root meristem should make it ideal for tracing meristem anlage defects defined by mutation.

MATERIALS AND METHODS

Plant growth conditions

For anatomical studies of the embryo, seeds of *A. thaliana* ecotype Columbia were sterilized in 5% sodium hypochlorite for 10 minutes and imbibed for 2 days at 4˚C in the dark in sterile water. Plants were grown on soil in a plant chamber at 22˚C, 75% humidity with a 16 hours light/8 hours dark cycle.

Sector analysis was performed on Landsberg *erecta* transgenic plants transformed with T-DNA from a plasmid pCL0461. This carries a 35S-*GUS* gene interrupted by an *Ac* element as described in Lawson et al. (1994), except that the *Ac* element was in the opposite orientation with respect to the direction of transcription from 35S-*GUS*. Seeds of two primary transformants carrying a single T-DNA insert based on segregation ratios were propagated on soil. F_2 lines homozygous for the T-DNA insert were selected. F_3, F_4 and F_5 seeds were bulked under controlled conditions as described above. Seeds used for analysis were sterilised, imbibed and allowed to germinate on plates containing 0.8% Duchefa plant agar in water. Plates were incubated for 4 days in a near vertical position using conditions described above.

For the [^{3}H]thymidine incorporation studies, ecotype Columbia seeds were sterilized, imbibed and allowed to germinate on plates containing 1× Murashige and Skoog (MS) salt mixture and 0.5 g/l 2-(N-morpholino) ethanesulfonic acid (MES), pH 5.8, in 0.8% Duchefa agar and 2 μCi/ml [^{3}H]thymidine (TRK.418,46 Ci/mmol; Amersham).

Selection of embryonic sectors

4 days after germination, seedlings were treated with heptane for 10 minutes, and stained for 8-16 hours at 37˚C in a solution containing 0.5 mg/ml Xgluc (Biosynth AG) dissolved in n-dimethyl-formamide, 0.1% Triton X-100, 0.5 mM $K_4Fe(CN)_6.H_2O$, 0.5 mM $K_3Fe(CN)_6$, and 50 mM sodium phosphate buffer, pH 7.2. 150,000 Stained

seedlings from 3 subsequent generations of transgenic plants were screened using a Zeiss SV 6 binoculair microscope. After estimation of the overall excision frequency, two categories of seedlings were selected for analysis.

(1) Seedlings carrying sectors that spanned one or more seedling regions (i.e. hypocotyl, intermediate zone, meristematic root) regardless of the position of the sector ends; and (2) seedlings with sectors covering half or more of a complete region with at least one sector end near the boundaries defined by sectors of a larger class. Selected seedlings were fixed for 3 hours in 1% glutaraldehyde, 4% formaldehyde in 50 mM sodium phosphate buffer, pH 7.2. Subsequently seedlings were rinsed in same buffer and dehydrated to 100% ethanol in a graded series.

Light microscopy

In order to study embryo development, growing siliques were harvested from soil-grown plants and dissected under a binocular microscope. Embryos were fixed for 3 hours in a solution containing 1% glutaraldehyde and 4% formaldehyde in 50 mM sodium phosphate buffer, pH 7.2. Subsequently the embryos were rinsed in the same buffer and pre-embedded in 1% agarose (Gibco). Dehydration followed in gradual steps: 10%,30%,50%,70%,90% and 2× 100% ethanol. Infiltration and embedding in Technovit 7100 (Kulzer, Hereaus) was performed as instructed by the manufacturer. 2-3 μm sections were made on a Reichert-Jung 1140 rotary microtome carrying a disposable Adamas steel knife. Sections were stained with 2.5% Astra blue (Merck) dissolved in 0.5 M sodium phosphate buffer, pH 4.4, at 50˚C for 8 minutes and photographed on a Zeiss Axioskop using Kodak Technical Pan film.

Seedlings carrying a sector selected for analysis were fixed and dehydrated as above. Technovit 7100 was infiltrated for 1 minute. The seedlings were then transferred to the following construction: celluloid transparency (Amovis), double-sided tape, transparency, double-sided tape. In the latter three layers a central region was excised to contain the seedling. Subsequently the seedlings were added in Technovit 7100 solution and the central region was covered by another transparency. Upon overnight polymerisation at room temperature a plastic platelet containing the seedling was obtained. In order to section embedded seedlings in the platelet, the celluloid sheet material was removed and the platelet was cut to allow transverse or longitudinal sectioning of relevant seedling regions. These platelets were re-embedded allowing simultaneous sectioning of 5 platelets. Cross sections of 5 μm or longitudinal sections of 4 μm were made. Sections were stained with 0.05% Ruthenium Red (Sigma) in distilled water for 10 minutes at room temperature. The platelets carrying the seedlings, and the sections were photographed on a Zeiss Photomikroskop III using Kodak Ektar 25 film. The longitudinal extent of sectors was recorded from the platelet photographs and in some cases directly from seedlings preserved in 70% ethanol. The radial extent of sectors was always determined on succesive cross sections of seedlings embedded in platelets, to minimize possible misinterpretations caused by diffusion.

For the visualisation of chlorophyll content, seedlings were placed in water between coverslips and photographed with a Zeiss Axioskop measuring fluorescence >515 nm after excitation at 490 nm using Kodak Ektachrome 400 film.

Image processing

In photographs of 12 serial sections of the root, depicted in Fig. 4A, labelled nuclei that could be distinguished by eye were manually positioned on drawings of the root section circumference. The manual marks were camera-recorded and superimposed using the IBAS image analysis system (Kontron Zeiss, Eching, Germany), resulting in the two-dimensional projection shown in Fig. 4B.

To quantify the grains per nucleus, the IBAS image analysis system was used with an adapted computer program. As the density of the silver grains in most cells was so high that clusters of grains had been formed, grain counting was rejected as a method for measuring the amount of radioactivity in favor of measuring the area. Slides containing the set of serial sections were microscopically examined (objective 100× oil immersion, projective 2.0×), and scanned with a Panasonic b/w CCD camera type WC-CD50, digitized 4 times and averaged to improve signal to noise ratio (frame size 640×512 pixels; 256 grey levels; pixel size 0.051×0.054 μm). The strategy of the program can be summarized as follows. To improve the edges of the silver grains, the contours in the image were enhanced. Subsequently the area of a nuclear section of interest was indicated interactively. Since the grey level of the background in each image can vary, semi-automatic selection of the silver grains demanded the application of a so-called dynamic discrimination. This method operates with a threshold that is dependent on the grey level of the local neighbourhood and was carried out in two steps for separate and clustered grains respectively. The resulting selections were combined. The result was compared with the online camera image, allowing corrections. The total area of selected grains was then measured. If a nucleus spanned more serial sections, the grain area values of all serial sections of the nucleus were summed. The program which performs the above mentioned operations can be made available by M. T. on request.

RESULTS

Embryonic development of the *Arabidopsis* root: histological analysis

We investigated embryonic anatomy to explore the cell lineage of the proposed initials within the root promeristem. Early cleavage patterns of the *Arabidopsis* embryo have been well documented (Mansfield and Briarty, 1991; Jürgens and Mayer, 1994). In summary, the zygote undergoes asymmetric cleavage to form a terminal and a basal cell. The terminal cell forms the *embryo proper*. The basal cell forms the various suspensor cells and the hypophyseal cell. This uppermost derivative of the basal cell participates in embryo development by contributing to the root pole.

Fig. 2 depicts *Arabidopsis* embryo development from globular stage onwards. The early globular *embryo proper* is split into an upper tier (ut) and a lower tier (lt) caused by the first transverse division of the terminal cell (Fig. 2A). At the triangular stage, transverse divisions have subdivided this lower tier (Fig. 2B). The resulting upper subtier forms a small zone of cells extending into the abaxial cotyledon shoulder at later stages. The basal subtier, termed ‘low lower tier’ (llt), consists of elongated procambium cells (PC) and one layer of protoderm (PD) and ground meristem (GM) each two cells high (Fig. 2B). The upper wall of the procambium cells marks the position of the llt boundary throughout the heart stage (Fig. 2B,D-F, open arrowhead). The activity of the cotyledon primordia frequently causes an indentation of the protoderm, that serves as a peripheral marker for the llt upper boundary throughout the heart stage (Fig. 2C-F, open arrow). At early heart stage the upper ground meristem cell layer is doubled by periclinal divisions (Fig. 2C, closed arrowhead).

Ongoing transverse divisions occur in the protoderm as well as in the procambium cells (Fig. 2D, open circle). At late heart stage the llt region becomes further subdivided when the lowest of the protoderm cells becomes recognisable as a root meristem initial by the characteristic periclinal division giving rise to the lateral root cap (Fig. 2E, LR1; Dolan et al., 1993). This defines the neighbouring llt-derived cells as root cortex

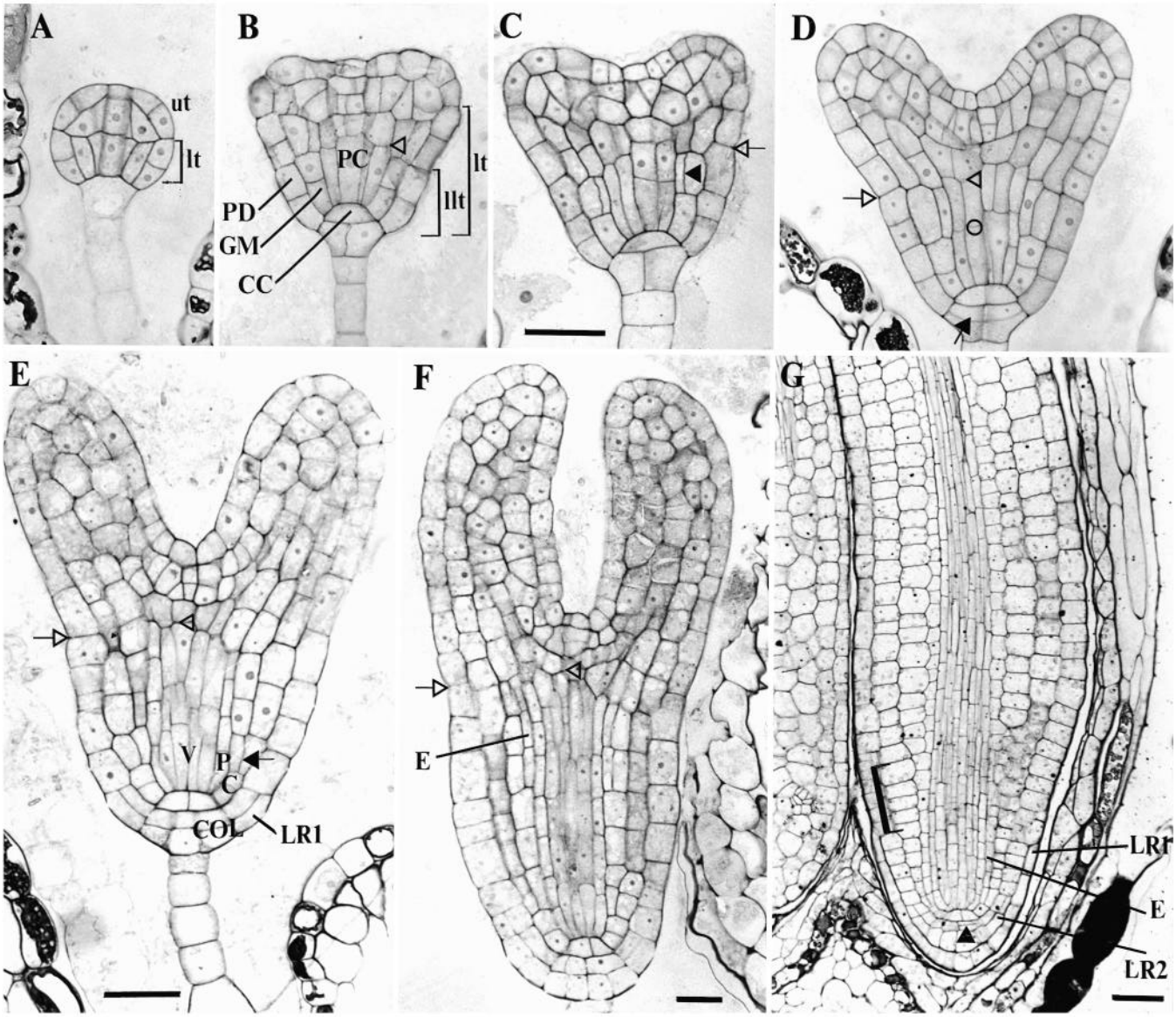


Fig. 2. Construction of the root promeristem during embryogenesis. Median longitudinal, Astra-blue stained sections. Stage numbers are according to Jürgens and Mayer (1994). (A) Early globular, stage 8; (B) triangular, stage 11; (C) early heart, stage 12; (D) mid heart, stage 13; (E) late heart, stage 14; (F) mid torpedo, stage 16; (G) mature embryo, stage 19. Open arrowhead: apical wall of 4th tier procambium. Open arrow: protoderm indention. Closed arrows, arrowheads and open circle: cell walls marking divisions as indicated in the text; ut, upper tier; lt, lower tier; llt, lower region of lt; PD, protoderm; PC, procambium; GM, ground meristem; CC, central cell; VP, vascular primordium; C, cortex; COL, columella; LR1, 1st lateral root cap layer; E, endodermis; LR2, 2nd lateral root cap layer. Bar, 25 μm.

(C), pericycle (P), and vascular (V) initials. Like the epidermis intials, root cortex initials have already produced a daughter cell. The ground meristem cells apical to the root cortex initial and its daughter perform periclinal divisions (Fig. 2E, closed arrow). Therefore, at late heart stage the llt ground meristem can be divided in four subtiers. The basal subtier is defined as the cortex initial, which has performed a transverse division. The apical three subtiers have doubled in the radial dimension by periclinal divisions. From histological data it cannot be decided whether these three double tiers define distinct embryonic regions fated to become hypocotyl or root. The inner layer of ground meristem within these subtiers forms the endodermis at torpedo stage by additional periclinal divisions (Fig. 2F).

The anatomy of the llt region where lateral root cap layers are present at torpedo and later stages can be explained by assuming division patterns of a set of initials as depicted in Fig. 1 (Fig. 2F,G). In mature embryos a region above the uppermost lateral root cap cell can exist, which contains a single cortical cell layer and therefore has the typical root anatomy (Fig. 2G, vertical bar). This region appears not to be formed by divisions in the promeristem.

The contribution of the hypophyseal cell to the root promeristem as inferred from histology is as follows. At the triangular stage the hypophysis has cut off a lens-shaped cell (Fig. 2B, CC). The lower hypophyseal cell derivative divides to form an additional tier at late heart stage (Fig. 2D, closed arrow). The derivatives adjacent to the epidermis initial are

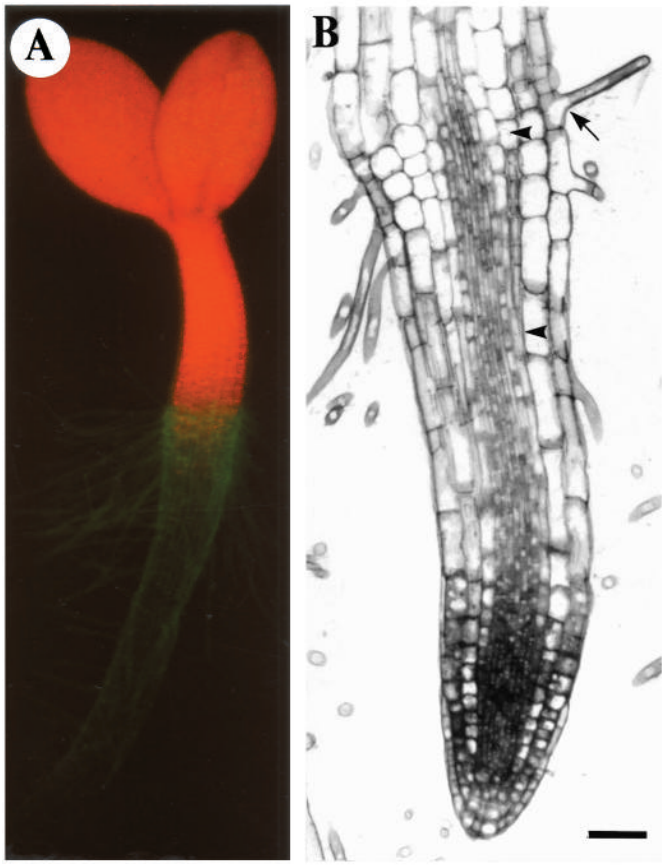


Fig. 3. Differentiation landmarks at the root-hypocotyl boundary. (A) Chlorophyll amount in a seedling 2 days after germination visualised by fluorescence. (B) Median longitudinal toluidine blue-stained section of *Arabidopsis* root 2 days after germination. The region between the two arrowheads contains two cortical cell layers. Arrowhead: cell within 2nd cortex layer; arrow: uppermost root hair-containing epidermis cell. Bar, 50 µm.

defined as columella initials (Fig. 2E, COL). At later stages these initials produce additional tiers of columella. The formation of a new cell wall for the last columella tier is marked (figure 2G, closed arrowhead).

Two distinct regions in the postembryonic root

Due to the absence of differentiation markers, embryo sections cannot prove where the root-hypocotyl boundary resides and hence whether the derivatives of the root promeristem are the only cells that will give rise to the postembryonic root. Fig. 3 displays differentiation characteristics of the root-hypocotyl boundary at the seedling stage. The cortex cells of the hypocotyl contain a substantial amount of chlorophyll. The transition to the low amount of chlorophyll in root cortex cells occurs within one cell (Fig. 3A). Epidermis cells abutting the most apical cortex cells with low chlorophyll content contain the most apical root hairs (in our description of orientation in the seedling we use 'apical' for structures located in the direction of the shoot pole, and 'basal' for structures located in the direction of the root pole, in accordance with the terms used in description of embryo development). The spatial convergence of these two differentiation landmarks suggests a sharp border between root and hypocotyl cell types. Therefore

Fig. 4. The embryonic region of the root does not perform extensive postembryonic divisions. (A) Median longitudinal section of [3H]thymidine-labelled root 3 days after germination. Silver grains marking labelled nuclei are visible as black dots. Arrowheads indicate point of disappearance of 2nd cortex cell layer. Bar, 50 µm. (B) Superimposition of labelled nuclei observed in all serial sections of the root shown in A. (C) Graph representing the amount of label in the nuclei per total number of cells in the arbitrarily chosen zones depicted in B. Every dot represents the summed grain area of one nucleus. Mean values per zone indicated by vertical bars. See Materials and methods for details on the calculations.

we defined the seedling region above the most apical root hairs as hypocotyl, and the region at and basal to these cells as root. In roots, as defined by this border, two distinct regions are present with different cellular organisation. Fig. 3B depicts a typical median longitudinal section of a root 2 days after germination. At the basal end meristematic cells can be observed to which cell files of the root converge. However, at the boundary of root and hypocotyl it can be seen that a separate region exists. In this region two layers of cortex cells are present (Fig. 3B, arrowheads). Epidermis cells in this region form root hairs (arrow). It is noteworthy that epidermis and cortex cells in this region elongate to a lesser extent than cells at more apical and basal positions. The height at which the second cortex layer disappears is somewhat variable (lower arrowhead). In the seedling root this region encompasses a column of 5.5±0.8 epidermis and 6.0±1.1 cortex cells (n=11).

In conclusion, a region with doubled cortex cell layer between the derivatives of the root meristem and the hypocotyl acquires root characteristics. We questioned whether this region arises by embryonic or postembryonic cell divisions. The 'intermediate' region cannot be recognized in the embryo for it lacks characteristics by which it can be distinguished from hypocotyl at that time. Therefore a distinct embryonic origin of this region cannot be proved by anatomical analysis. An alternative scenario can account for the formation of this region, namely the first (oldest) cortical derivatives of the promeristem perform a periclinal division after germination to generate a region with a double cortex layer which is not part of the hypocotyl. We performed in vivo [^{3}H]thymidine labellings to establish whether such divisions occur after germination. Seedlings were germinated on medium containing [^{3}H]thymidine and the incorporated label was detected on sections by autoradiography. A typical median longitudinal section of a root, 3 days after germination, is shown in Fig. 4A. The end points of the second cortical cell files are indicated by arrowheads. Labeled nuclei are largely confined to the part of the root containing only one cortex cell layer. Fig. 4B shows superimposed dots representing all labelled nuclei in 12 serial sections of this root. Few labelled nuclei are detected in the intermediate region of the root covered by the serial sections (zone 5 in Fig. 4B). In the intermediate region, nuclei can be found that are not labelled, in contrast to the meristematic region in which every nucleus is labelled (data not shown). However, these results can be influenced by size differences of cells and nuclei in the different regions of the root. Therefore we determined the amount of grains overlying labelled nuclei in different zones of the root. The grain area over serial sections of nuclei was summed. According to this analysis, nuclei in the intermediate region were weakly labelled, in contrast to nuclei in the meristematic zone (Fig. 4C). Henceforth no evidence for systematic cell divisions in this part of the root was obtained. We interpreted this as evidence against the hypothesis that the intermediate region arises through postembryonic divisions, from cells derived from the promeristem. Furthermore, because of the virtual lack of cell divisions, the cell numbers of the intermediate region in the postembryonic root have to equal the cell numbers of the part of the root that is already present in the mature embryo. This region cannot be derived from the promeristem during embryogenesis because all progeny of the promeristem to the inside of the lateral root cap in the embryo contains only one layer of future root cortex cells.

In some instances the lateral root cap can be observed to be at a variable distance basal to the position where the second cortex layer of the interzone resides. This observation should be treated with caution since lateral root cap cells slough off the root upon ageing. Nevertheless the presence of a small embryonic region of the root with a single cortex cell layer that is not formed by the proposed initials can also be inferred from sections of mature embryos (Fig. 2G, vertical bar).

Clonal analysis by transposon excision

Despite the apparent ease in determining cell division patterns in plant cells, which are thought to have only a limited capacity to move relative to one another, our anatomically derived view on root morphogenesis needs proof from other lines of evidence. Clonal analysis is a means of investigating whether distinct embryonic cell tiers are specified at an early stage to elaborate the meristem initials, the embryonic root and hypocotyl in the embryo. Blue staining sectors in transgenic *Arabidopsis* seedlings containing the construct depicted in Fig. 5 were analysed for this purpose. Such sectors encompass all the daughters of any cell in which excision of the maize *Ac* transposon restores expression of a β-glucuronidase gene driven by the CaMV 35S promoter.

Since it is assumed that *Ac* excision can occur at random at all developmental stages, we selected sectors sufficiently large to represent embryonic excisions (see Materials and methods for selection criteria) and mapped the end points of these sectors on to the seedling structure. The underlying rationale was that the end points of the largest sectors would demarcate the earliest embryonic divisions and end points of sectors of decreasing size would mark later subdivisions. The frequencies of large sectors useful for our analysis are listed in Table 1. Large embryonic sectors are rare compared to the overall sector frequency. This frequency distribution is expected if the amount of cells available for an excision event at a certain developmental stage is an important parameter for the observed excision frequency. We selected transgenic lines with a low enough probability of excision per cell per seedling to safely assume that the vast majority of the large embryonic sectors used in our analysis were the result of a single excision event. Seedlings carrying such embryonic sectors are shown in Fig. 6.

The end points of 162 large sectors were analysed and the sectors are depicted schematically in Fig. 7. Sectors ends, of which the precise cellular position was determined are represented by completely closed bars. All sectors indicated that sector ends clustered at preferred positions in the seedling. Using the precisely determined ends a variation in the position of sector ends around preferred positions was observed (Fig. 7). The end points of 10 root sectors, ending precisely in an initial, constituted an exception, in that they were not variable (Fig. 7A,B,E,F, closed bars). However, suppression of marker gene expression was often observed in the meristematic zone, especially in vascular tissue (Fig. 6A-C). Hence, we had to include sectors that did not end in the initials in the 'not accurately determined' group (Fig. 7B,E,F, open-ended bars), preventing any analysis of variation.

The largest sector found in our study spanned the abaxial cotyledon shoulder, the axial region between the hypocotyl and the shoot meristem where the vascular bundles separate, the hypocotyl, and the root without the central cells and columella

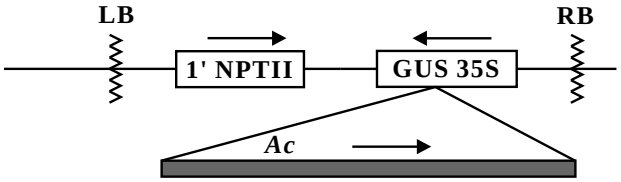


Fig. 5. T-DNA construct in transgenic plants used for clonal analysis. Binary vector carrying *Ac* in a 35S-*GUS* gene fusion. The *Ac* element in plasmid pJJ4361 (Jones et al., 1992) was excised as a *Sal*I-*Sst*I fragment. It was cloned into a plasmid pSLJ732 (Jones et al., 1992) carrying a 35S-*GUS* marker, at *Sal*I and *Xho*I sites immediately downstream of the transcription start of the 35S promoter and lying in a TMV Ω 5′ untranslated leader.

(Fig. 7A). This sector extended in all tissues and spanned roughly 1/4 of the seedling circumference in hypocotyl and root. In the cotyledon shoulder the sector was centered on half the area of the cotyledon and extended in all tissues including the central vascular bundle (data not shown). Based on the length and width, the upper end of this sector was taken to represent the position of the first transverse division within the *embryo proper*, in the seedling (Fig. 2A, division plane between ut and lt). Our histological observation supports the idea that the plane of the first transverse division within the *embryo proper* extends into the cotyledons (Fig. 2B-D).

The next largest sectors spanned the complete root and hypocotyl just below the separation of the vascular strands (Fig. 7B). The upper end of these sectors were interpreted to represent the next subdivision of lt cells, creating the llt region. The position of this boundary was corroborated by the existence of complementary cotyledon shoulder sectors; these all approached the same boundary from the apical end (Fig. 7C). The next large sectors spanned either complete hypocotyl or complete root (Figs 6A-C,E, 7D,E). Therefore the next subdivision of the ll tier was interpreted to be around the root/hypocotyl boundary. Subsequent division of the hypocotyl region yielded sectors spanning approximately half of the hypocotyl (cf Fig. 7D). The basal hypocotyl sector ends were plotted in the histogram shown in Fig. 8A. The observed distribution of sector ends allowed calculation of the mean sector boundary at position 0.2±1.9 (n=48), where cell 0 represents the uppermost root hair-containing cell. From these data the averaged position of the ll tier subdivision in the seedling was estimated to coincide with the root/hypocotyl boundary defined by the uppermost root hair-containing cell (Fig. 7). This calculated position was then used to select complementary root sectors that approached from the basal end of the seedling. Such sectors fell into two distinct classes. A class of long sectors existed, which extended from this boundary into the root meristem initials (Figs 6A,C, 7E). A second class of short sectors spanning the intermediate region also approached the calculated boundary (Figs 6D, 7G). From this class, only sectors spanning 4 or more cells were used for analysis of lower sector ends, to minimise the possibility that the end point resulted from a later subdivision. These sectors had an averaged basal boundary position at 8.7±2.8 (n=57) epidermal cells basal to the most apical root hair-containing cell (Fig. 8B). Therefore, sectors spanning the intermediate region on average ended more basal than the position where the characteristic second cortical cell layer disappears. Henceforth the point of disappearance of this additional layer does not form a clonal boundary. Noteworthy, only one sector with the apical end at the root/hypocotyl boundary was longer then 12 cells without extending into the root meristem (Fig. 8B).

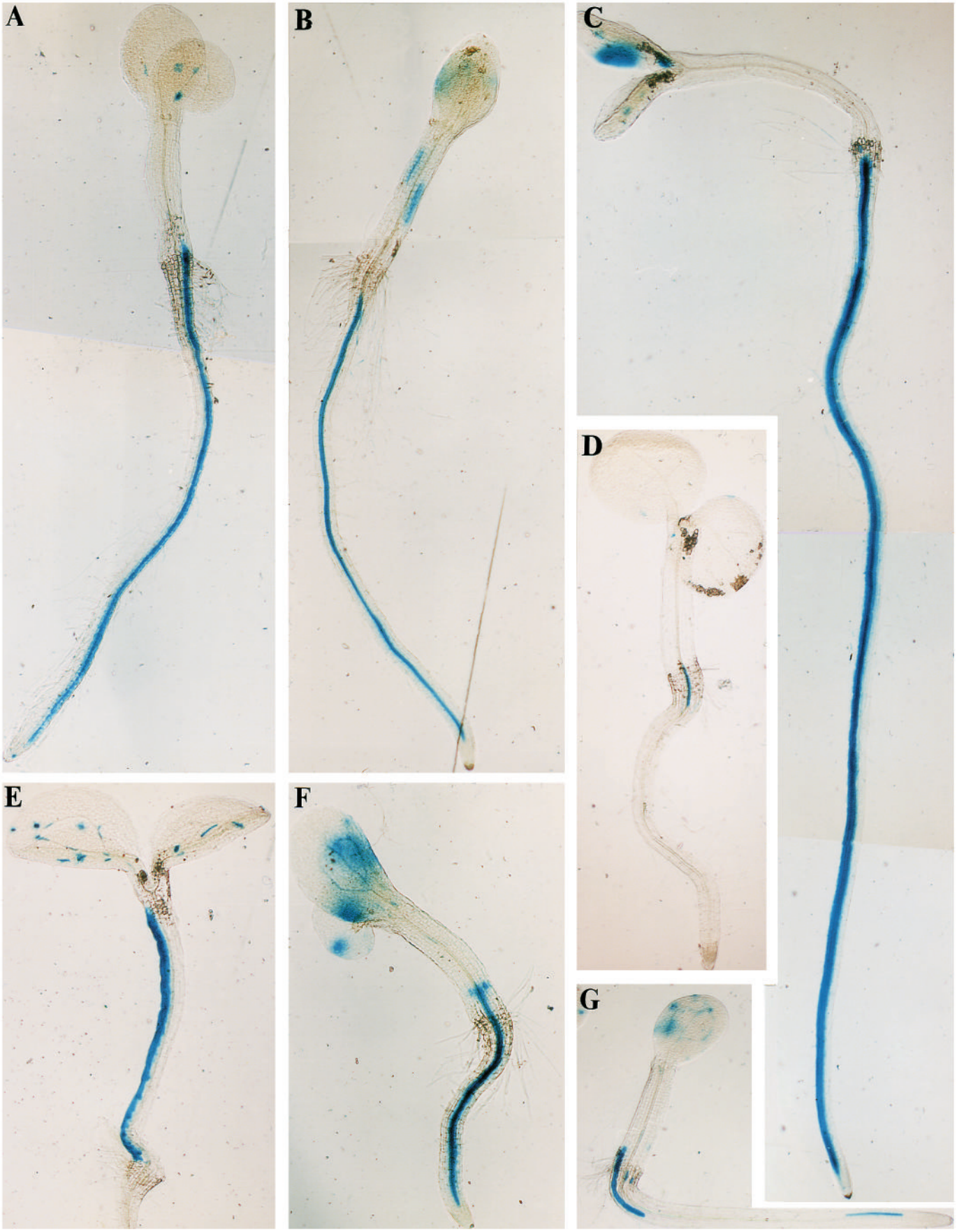
Table 1. Sector frequency comparison

	Total	Embryonic	Analysed
Hypocotyl	7.7%	0.3% (450)*	48
Interm. zone	8.4%	1.2% (1800)†	57
Root	12.1%	0.2% (300)‡	44
lt		1§	1
llt		2¶	2
Quiescent centre		1**	1
Cotyledon shoulder		9	9

*Sector spanning complete or half hypocotyl
†Sector four or more cells long
‡Sector spanning region in or near initial to near intermediate zone.
§Sector spanning cotyledon shoulder, hypocotyl and root
¶Sector spanning complete root and hypocotyl
**Sector encompassing all four quiescent centre cells
Numbers and frequencies represent sectors found in 150,000 seedlings. The frequency of hypocotyl, intermediate zone, and root sectors was estimated by counting all sectors in 5071 seedlings in 17 separate samples. From the frequency the number of embryonic root, hypocotyl, and intermediate zone was calculated (number in parentheses).

We interpreted these observations as follows. The complete root sectors represent the counterparts of the hypocotyl sectors resulting from the first llt subdivision. The basal end points of the small intermediate zone sectors demarcate the second subdivision of the resulting root region. Subsequently, smaller sectors extending completely into the initials represent different time points of excision from the meristem initials (Fig. 7F). Sectors disconnected from the meristem but not approaching the average root-hypocotyl boundary represent excisions in the descendants of the meristem initials. Such sectors occurred frequently (cf. Table 1) but they were excluded from further analysis since they provided no information on the position of embryonic divisions in the seedling.

Inspection of all seedlings carrying embryonic sectors limited to the root showed that all sectors were restricted to one of the major tissue types epidermis, cortex/endodermis, and pericycle/vascular bundle (cf Fig. 6). This observation is in accordance with our previously proposed model, stating that initials for the major tissue types are separated during embryogenesis and each produce a restricted set of daughter cell types. Estimation of the width of root sectors extending into the root meristem allows a more precise reconstruction of the apparent cell numbers of initials of the different tissue types at the developmental stage when excision took place. We sectioned seedlings carrying root sectors (as depicted in Fig. 7E,F) and compared the sector width with the radial pattern in the lower ll tier proposed to form the meristematic initials. Fig. 9 depicts cross sections showing the radial pattern of the proposed meristem initials at the triangular and mid torpedo stage, as well as the width of typical root sectors. At the early heart stage, the protoderm and ground meristem cell layers are already at or close to the average cell number found in mature root meristems (Fig. 9A, cf Dolan et al., 1993). However, between four and eight prospective pericycle and four vascular cells are present at that stage. At the torpedo stage the prospective root pericycle and vascular initials approach the cell numbers observed in the postembryonic meristem (Fig. 9B).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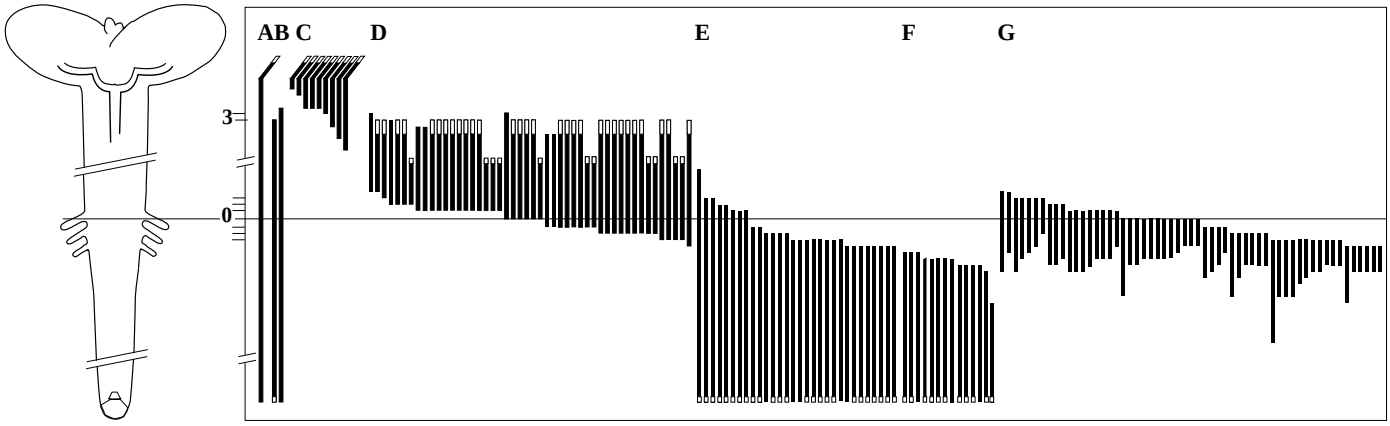

Fig. 7. End points of sectors resulting from embryonic excision. Every sector is represented by a bar. The end points of the bars represent the position of the sector ends in the schemetically drawn seedling. Black-ended bars indicate that the exact cellular location of a sector terminus was determined; white-ended bars indicate that the sector end was estimated. (A) Sector demarcating lt/ut division; (B) root plus hypocotyl sectors; (C) cotyledon shoulder sectors; (D) hypocotyl sectors; (E) root sectors with apical ends overlapping basal ends of hypocotyl sectors; (F) root sectors with apical ends disconnected from basal ends of hypocotyl sectors; (G) 'intermediate zone' sectors. On the vertical axis the bars represent cell walls of adjacent epidermis cells. 0: lower wall of the first root hair-containing epidermis cell; 3: third epidermis cell below junction of cotyledon vascular strands.

Embryonic sectors in the root epidermis and cortex/endodermis were always 1 cell wide (n=10+10), consistent with the idea that the cell number of the initials at the time they are set apart equals the cell number of the postembryonic initials. Cortex/endodermis sectors ended on shared initials consistent with their common origin in the promeristem (Fig. 9C,D). Pericycle sectors as well as vascular sectors were found extending to 1/4 of the circumference and were therefore consistent with a cell number of 4 at the time of excision (Fig. 9E,F). Smaller vascular sectors were also found but they always contained more then one cell in the radial dimension (data not shown). We interprete these sectors to represent slightly later excisions from the initials, at a stage in which the radial pattern is further elaborated.

In conclusion, sectors of the complete *Arabidopsis* root and hypocotyl regions as well as sectors spanning only a part of the root or hypocotyl revealed preferred locations of ends, and root sectors revealed restricted contribution of different initials to tissue types. The positions in the seedling of the first and second division planes of llt cells enable us to make a map in which cells, at the heart stage, are associated with root or hypocotyl fate. Such a fate map is depicted in Fig. 10. The first and second subdivisions of the ll tier, at heart stage, divide the seedling into four regions defined by the sector ends. The variation in sector ends is represented in the fate map by the grey lines which indicate the amount of variability in the position of a particular subtier boundary in the seedling. At the early heart stage three distinct regions are visible in the ll tier. The apical region will form most cells of the hypocotyl; the middle region, most of the intermediate region of the root; the basal region the epidermis, cortex, and stele initials of the meristematic root. The first subdivision after the splitting of prospective root and hypocotyl region is drawn in the root region (Fig. 10A). If this subdivision first occurred in the hypocotyl region, vascular sectors spanning all of the root and half of the hypocotyl would be frequent. The only two sectors we found that could be a consequence of such a division pattern after at least one further subdivison are depicted in Fig. 7F,G. These sectors therefore occur with very low frequency compared to the ones with 'regular' ends.

Basal end points of complete root sectors (or larger sectors spanning root and hypocotyl) never intruded into the columella and central cell region of the root meristem. The only embryonic sector in the hypophyseal cell region that we identified encompassed all four central cells but did not extend into the root. These observations support histological data and pinpoint the hypophyseal cell derivatives as an early restricted lineage.

Fig. 6. Seedlings carrying sectors resulting from embryonic excision. (A) Total root; (B) meristematic root ; (C) meristematic root; (D) embryonic root (E) hypocotyl; (F) hypocotyl + root; (G) hypocotyl + root. Complete root sectors show suppression of marker gene expression in the meristematic zone and are overstained to visualise stain in the initial cells.

DISCUSSION

Clonal analysis of *Arabidopsis* root formation by marked transposon excision

We have applied both histological techniques and the analysis of sectors originating around the heart stage of embryogenesis to follow the development of the primary root in *Arabidopsis*. Histological data predict a rigid pattern of cell division in the lower tier of the *embryo proper*, giving rise to root and hypocotyl. Previously, we proposed that a fixed pattern of cell divisions perpetuates in the meristematic zone after germination, to form the root (Dolan et al., 1993). The notion of rigid embryonic and postembryonic division patterns is fully consistent with the relatively fixed position of the end points of large sectors in our clonal analysis. The clonal analysis corroborates our earlier model concerning the onset of root meristem activity at the heart stage of embyogenesis. Furthermore, new information on the contribution of different embryonic tiers to root, hypocotyl and cotyledon shoulders of the seedlings is obtained.

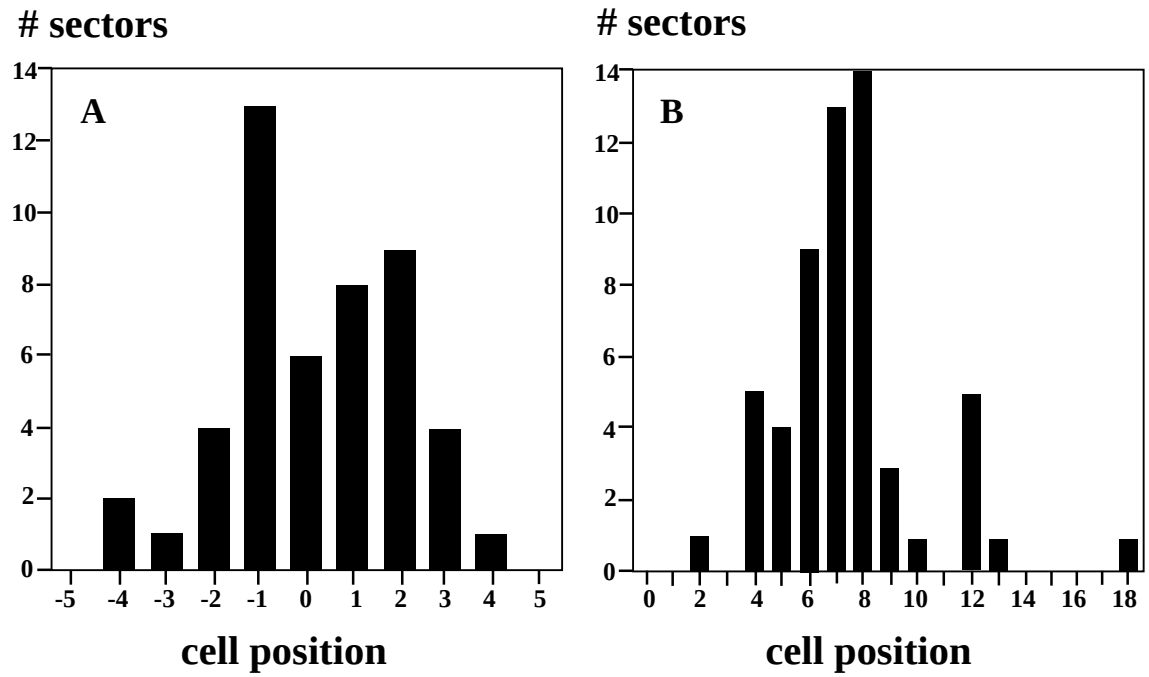

Fig. 8. Histograms showing distribution of sector ends demarcating first and second subdivision of ll tier. (A) Position of basal end points of complete or half hypocotyl sectors. (B) Position of basal end points of 'intermediate region' sectors with an apical end at the position of the first llt division. The position of sector ends was counted in epidermis cells below the anatomical boundary of root and hypocotyl. Cell 0: first epidermis cell forming root hairs. Negative numbers, hypocotyl cells; positive numbers, root cells.

Sufficient embryonic sectors are created by excision of the *Ac* transposable element in a β-glucuronidase gene driven by the CaMV 35S promoter in transgenic plants. Easily scorable sectors in all cell types of the *Arabidopsis* root and hypocotyl can be obtained, although suppression of *GUS* expression in the meristematic zone caused a problem in determination of meristematic sector ends. An important conceptual difference between clonal analysis, using transposon sectors, and the acute irradiation type sectors, is that the former sectors originate at any developmental stage while radiation sectors can be induced at specific stages (e.g. Poethig and Sussex, 1985; Poethig et al., 1986). The consequence for the analysis of clonal data is that the time point of excision must be inferred. Due to the relative invariance of the sector ends in root and hypocotyl, smaller sectors can be recognised as subdivisions of large sectors. However, if cell fates are more variable the uncertainty about the time of excision may seriously hamper clonal analysis. We determined the embryonic stage at which excisions had occurred using only one assumption: 'the first subdivision of the terminal cell of the zygote separates hypocotyl, root and cotyledon shoulder from the shoot meristem anlage and the cotyledons'. This assumption is based on our histological observations and we positioned the single largest sector accordingly. The necessity to make this starting assumption is not fundamental, but a consequence of the low frequency of sectors originating at or before the globular stage of embryogenesis. Upon positioning of the largest sector, analysis of cotyledon shoulder, hypocotyl and root sectors of decreasing size allowed the definition of preferred boundaries in the seedling representing subsequent apical-basal divisions of the lower tier of the embryo. Superimposition of the resulting scheme for subdivision of the seedling on the anatomy of the heart stage embryo leads to the fate map shown in Fig. 10.

Mutations in several genes have been described that produce regional deletions in seedling root and hypocotyl (Mayer et al., 1991). Interestingly, studies on the development of mutant embryos indicate that the absence of both root and hypocotyl in seedlings homozygous for strong *monopteros* alleles is caused by an abbarent division pattern of the cell in the position of the hypophysis (Berleth and Jürgens, 1993). Therefore, there may be no *a priori* misspecification of all cell tiers which normally are fated to become root and hypocotyl. This observation indicates that the fate map we generated does not necessarily predict the embryonic origin of all regional deletions observed in seedlings, although it certainly can add to the understanding of deviations in embryo development that are actually observed.

Establishment of the root/hypocotyl boundary in the embryo

High rigidity and reproducibility of cell divisions in the embryo of many angiosperm species has been reported. In *Arabidopsis*, this rigidity seems perpetuated in the seedling root (Dolan et al., 1993). This poses the question of whether rigid cell division patterns in the embryo axis reflect the creation of developmental compartments. We chose to investigate the formation of the root/hypocotyl boundary as an example of such a compartmentalisation.

The majority of sectors spanning the complete root or hypocotyl terminated near, but not precisely at, the root-hypocotyl junction as defined by two cellular differentiation criteria. Our interpretation of these sectors is that the future root-hypocotyl boundary forms near, but not necessarily at, the

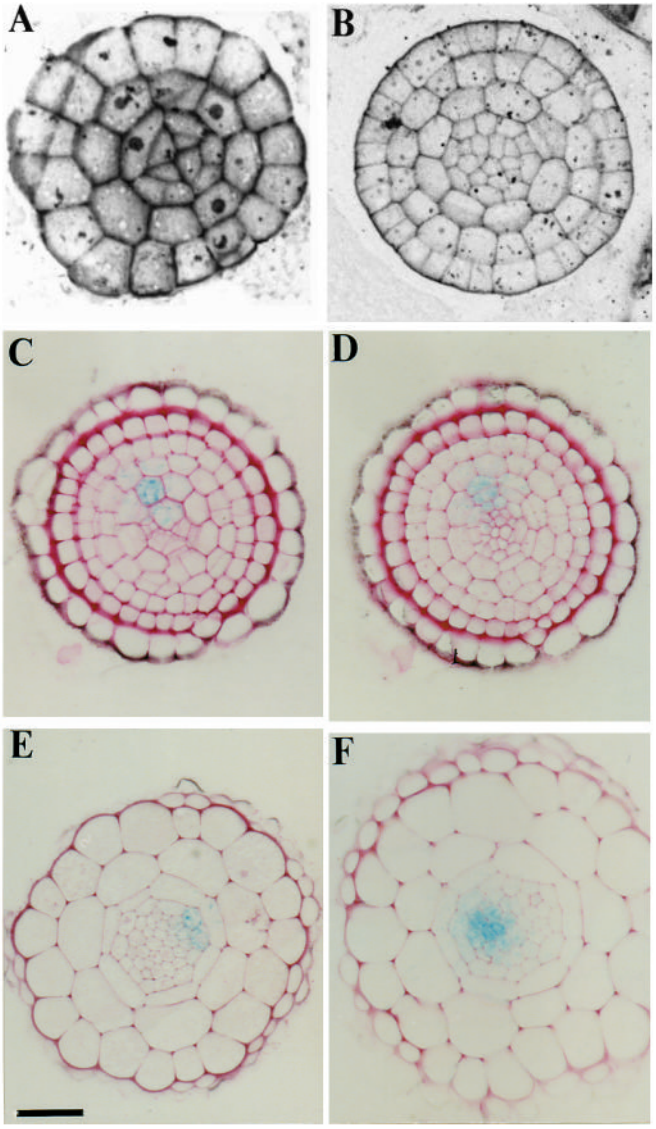

Fig. 9. Radial extent of embryonic root sectors as determinded from cross sections correspond to the anatomy of the early heart stage promeristem. (A) triangular/early heart stage embryo; (B) mid torpedo stage embryo; (C) seedling carrying root sector, sectioned at the initial. (D) Same seedling as in C, the plane of the section being above the initial showing clonal relation between cortex and endodermis cell files. (E) Seedling carrying complete root sector in pericycle; (F) seedling carrying complete root sector in vascular bundle. Cross sections of embryo (A,B) are Astrazol blue stained. Cross sections of X-gluc treated seedlings (C-F) are counterstained with Ruthenium Red. Bar, 50 μm.

first transverse division plane of cells in the ll tier (cf Fig. 2B). Occasionally observed sectors that grossly violated the compartment boundaries without spanning the total root and hypocotyl, further undermine the idea of any overriding importance for lineage relationships in specification of these embryonic regions. In general, examples of pattern formation in plants in which clonal relationships have been best investigated do not present evidence in favour of lineage restricted developmental potential. In maize, for example, the notion of tassel-restricted cell lineages (Coe and Neuffer, 1978) was not supported by a more elaborate sector analysis (Poethig et al., 1986; McDaniel and Poethig, 1988).

The fate map of the heart stage embryo depicts a cell tier destined to acquire root fate but not derived from the meristematic initials (cf Fig. 10). The average fate of this cell tier is to form the 'intermediate region' with two cortical cell layers that can be distinguished in the seedling root. However, the end points of sectors spanning this region show that this embryonic tier can also give rise to a small portion of the root with only a single cortical cell layer. These data are consistent with the frequent occurrence of a region in mature embryos containing a single cortical cell layer, but not flanked by lateral root cap cells and therefore not derived from the meristem initials. One of the main characteristics of the epidermis in this region is the rapid formation of root hairs with different spacing characteristics compared to the root hairs formed on epidermis cells derived from the meristem initials (B. S., data not shown; L. Dolan, personal communication). A region at the junction of root and hypocotyl with such characteristics has been noted in other plants and has been called the 'collet' (Eames, 1961). It is tempting to speculate that these observations imply the presence of a distinct, non-meristematic part of the root in many plant species.

Formation of the root meristem in the *Arabidopsis* embryo

We have proposed earlier, on the basis of histological data, that the root meristem initials in *Arabidopsis* are defined at the heart stage of embryogenesis. These initials are thought to perform the same division patterns in the later stages of embryogenesis as they do in the early stages of seedling development (Dolan et al., 1993; cf Fig. 1). The presence of seedlings carrying root sectors with end points in the initials and extending up to the intermediate zone or hypocotyl boundary is consistent with the model stated above. In this model it was proposed that epidermis and cortex initials produce two different cell types. The presence of sectors spanning both cortex and endodermis observed in our study and by Dolan et al. (1994), and sectors spanning both epidermis and lateral root cap (Dolan et al., 1994), lend further support to this model.

The radial extent of pericycle and vascular bundle sectors indicates that the radial pattern of the root meristem initials at the time of their activation equals the radial pattern of the proposed group of initials between heart stage and torpedo stage of embryogenesis. Completion of the radial pattern of the initials occurs at the torpedo stage. We conclude that initial cells, first discernible at the late heart stage of embryogenesis, indeed perform repetitive division patterns as previously proposed, but that pericycle and vascular initials perform several additional divisions between the late heart and the torpedo stages to complete the radial pattern observed in the postembryonic root.

Replacement of embryonic initials has been proposed by studies on plants with a longer life cycle than *Arabidopsis* (von Guttenberg, 1964). We have not been able to investigate fully the permanency of the initials defined during embryogenesis, due to the uncertainty of the excision time point in embryonic sectors. Sectors that do not extend into the meristem can arise in two different ways. They can be caused either by excisions in replaced initials, or by (post)embryonic excisions in derivatives of initials. The observed downregulation of the 35S

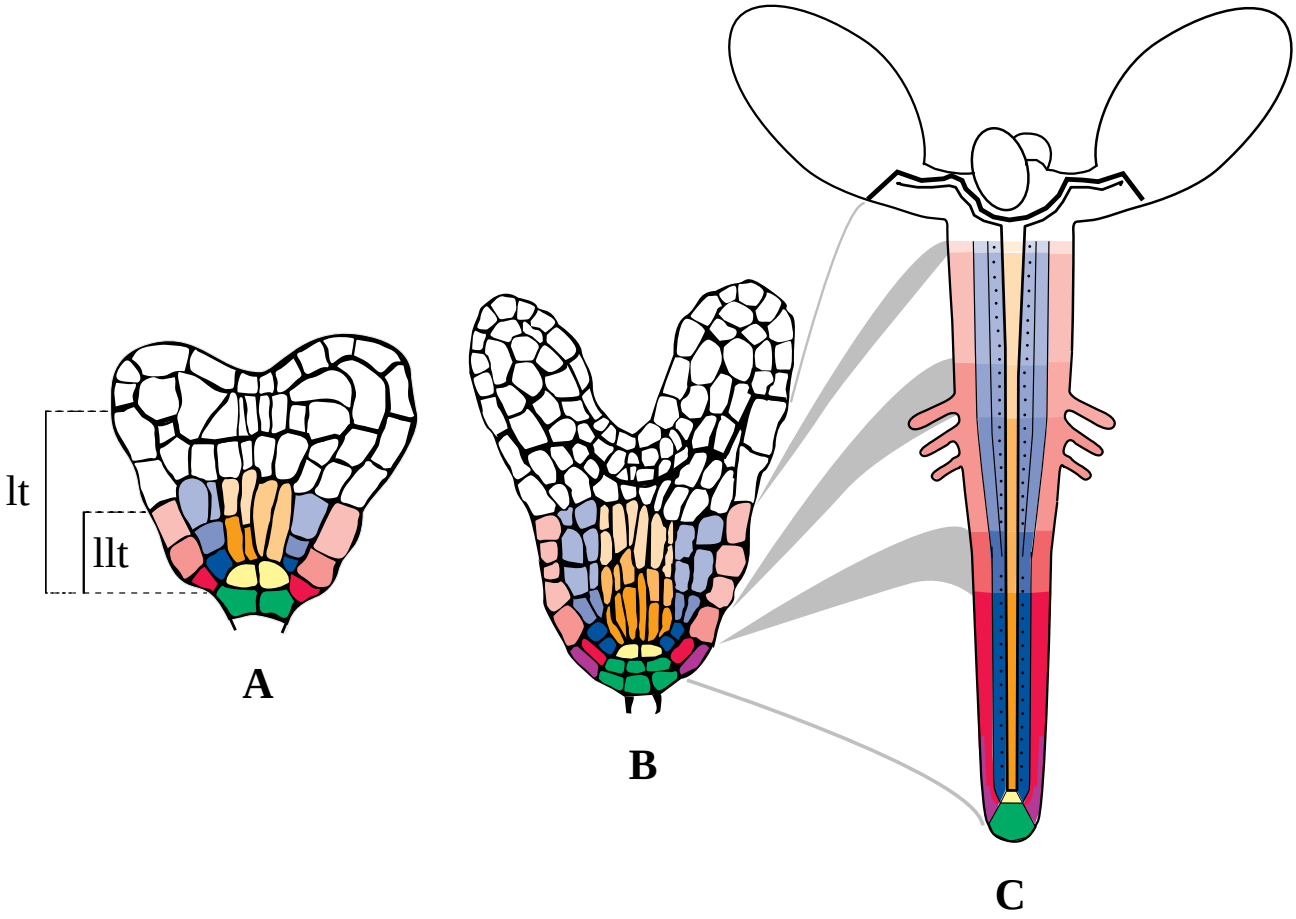

Fig. 10. Cell fates in the lt region. Corresponding lt regions in early heart stage embryo (A), late heart stage embryo (B) and seedling (C) are colour coded. In the case of cells that are not associated with a fixed destination, the mean value of the colour intensities of alternative destinations is depicted. The contribution of the different embryonic tiers to the seedling structure is indicated by colour as well as by the grey lines that indicate the variable position in the seedling of embryonic tier boundaries. The extent of the 'll tier' is depicted in A. The position of the ut/lt division plane at different developmental stages is represented by a bold line. Red, protoderm/epidermis; purple, lateral root cap; blue, ground meristem/cortex and endodermis; orange, vascular cambium/stele; yellow, quiescent centre; green, columella. The root meristem initials and their derivatives have the highest intensity of colour.

promoter in the meristematic zone further hampers analysis of initial replacement. Sectors spanning the complete embryonic root ending in the root meristem but not in the initials can be caused either by initial replacement or by insufficient staining. In principle, analysis of the frequency of complete root sectors at later stages of seedling development could circumvent this technical problem. However, due to cell death in the root seemingly related to secondary growth, continuous sectors cannot be scored (H. W. and B. S., unpublished data).

In summary, the sectors extending into the initials indicate the existence of 'permanent' initials in the limited time span of our observation, but we cannot determine the frequency of permanency versus replacement. It is noteworthy that no complete root sectors were found that extended into the central cell/columella region of the root. The one sector found in the central cells originated at or before the triangular stage and this sector likewise did not violate the boundary defined by the complete root sectors, nor did it show contribution of central cells to columella formation. These results imply a developmental restriction of the hypophyseal cell derivatives. The possible participation of hypophyseal daughter cells, e.g. quiescent centre cells, in the replacement of proximal initials would be a violation of this apparent restriction.

The rigid pattern of divisions of initials which has been inferred from our data can easily lead to the interpretation of 'histogens', containing initials with restricted developmental potential *sensu* Hanstein (1870). It is important to stress that our data on the separate cell lineage of epidermis, cortex and stele initials cannot be considered as evidence that cell lineage determines cell fate. The fact that derivatives of a cell acquire the same fate may be a simple reflection of the division patterns, which give all the descendants a similar position in an informational field without genetic restrictions of their developmental potential. Such an interpretation is in accordance with the observed interchangeability of tunica and corpus layers of the shoot meristem (Dermen, 1953; Stewart and Burk, 1970). An analysis that uses sectors with different growth characteristics, analogous to the *minute* based clonal analyses in *Drosophila* (Garcia-Bellido et al., 1976), may shed light on this issue.

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REFERENCES

Benfey, P. N., Ren, L. and Chua, N.-H. (1990). Combinatorial and synergistic properties of CaMV 35S enhancer subdomains. *EMBO J.* **9**, 1685-1696.

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.

Brumfield, R. T. (1943). Cell-lineage studies in root meristems by means of chromosome rearrangements induced by X-rays. *Am. J. Bot.* **30**, 101-110.

Clowes, F. A. L. (1954). The promeristem and the minimal constructional centre in grass root apices. *New Phytol.* **52**, 48-57.

Clowes, F. A. L. (1959). Reorganization of root apices after irradiation. *Ann. Bot.* **23**, 205-210.

Clowes, F. A. L. (1961). *Apical Meristems.* Oxford: Blackwell.

Coe, E. H. Jn and Neuffer, M. G. (1978). Embryo cells and their destinies in the corn plant. In *The Clonal Basis of Development* (ed. S. Subtelny and I. M. Sussex), pp. 113-129. New York: Academic Press.

Davidson, D. (1961). Mechanisms of reorganization and cell repopulation in meristems in roots of *Vicia faba* following irradiation and colchicine. *Chromosoma* **12**, 484-504.

Dermen, H. (1953). Periclinal cytochimeras and origin of tissues in stem and leaf of peach. *Amer. J. Bot.* **40**, 154-168.

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.

Dolan, L., Duckett, C., Grierson, C., Linstead, P., Schneider, K., Lawson, E., Dean, C., Poethig, S. and Roberts, K. (1994). Clonal relations and patterning in the root epidermis of *Arabidopsis*. *Development* **120** 2465-2474.

Eames, A. J. (1961). *Morphology of the Angiosperms*. New York: McGraw-Hill.

Furner, I. J. and Pumfrey, J. E. (1992). Cell fate in the shoot apical meristem of *Arabidopsis thaliana. Development* **115**, 755-764.

Furner, I. J. and Pumfrey, J. E. (1993). Cell fate in the inflorescence meristem and floral buttress of *Arabidopsis thaliana. Plant J.* **4**, 917-931.

Garcia-Bellido, A., Ripoll, P. and Morata, G. (1976). Developmental compartmentalization in the dorsal mesothoracic disc of *Drosophila*. *Dev. Biol.* **48**, 132-147.

Hanstein, J. (1870). Die Entwicklung des Keimes der Monocotylen und der Dikotylen. *Bot. Abhandl. (Bonn)* **1**, 1-112.

Irish, V. F. and Sussex, I. M. (1992). A fate map of the *Arabidopsis* embryonic shoot apical meristem. *Development* **115**, 745-753.

Jegla, D. E. and Sussex, I. M. (1989). Cell lineage patterns in the shoot meristem of the sunflower embryo in the dry seed. *Dev. Biol.* **131**, 215-225.

Jones, J., Shlumukov, L., Carland, F., English, J., Scofield, S., Bishop, G. and Harrison, K. (1992). Effective vectors for transformation, expression and assaying transposon activity in transgenic plants. *Transgenic Research* **1**, 285-297.

Jürgens, G. and Mayer, U. (1994). *Arabidopsis*. In *A colour Atlas of Developing Embryos* (ed. J. Bard), pp. 7-21. London: Wolfe Publishing.

Kuras, M. (1980). Activation of embryo during rape (*Brassica napus* L.) seed germination. II. Transversal organisation of radicle apical meristem. *Acta. Soc. Bot. Pol.* **49**, 387-395.

Lawson, E., Scofield, S., Sjodin, C., Jones, J. and Dean, C. (1994). Modifications to the 5′ untranslated leader region of the maize *Activator* element lead to increased activity in *Arabidopsis*. *Mol. Gen. Genet.* (in press).

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

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.

McDaniel, C. N. and Poethig, R. S. (1988). Cell-lineage patterns in the shoot apical meristem of the germinating maize embryo. *Planta* **175**, 13-22.

Natesh, S. and Rau, M. A. (1984) The embryo. In *Embryology of Angiosperms.* (ed. B. M. Johri), Springer-Verlag.

Poethig, R. S. (1987). Clonal analysis of cell lineage patterns in plant development. *Amer. J. Bot.* **74**, 581-594.

Poethig, R. S., Coe, E. H. Jr. and Johri, M. M. (1986). Cell lineage patterns in maize embryogenesis: a clonal analysis. *Dev. Biol.* **117**, 392-404.

Poethig, R. S. and Sussex, I. M. (1985). The cellular parameters of leaf development in tobacco: a clonal analysis. *Planta* **165**, 170-184.

Steeves, T. A. and Sussex, I. M. (1989). *Patterns in Plant Development.* Cambridge: Cambridge University Press.

Stewart, R. N. and Burk, L. G. (1970). Independence of tissues derived from apical layers in ontogeny of the tobacco leaf and ovary. *Amer. J. Bot.* **57**, 1010-1016.

Tykarska, T. (1979). Rape embryogenesis. II. Development of the embryo proper. *Acta Soc. Bot. Pol.* **48**, 391-421.

von Guttenberg, H. (1964). Die entwicklung der Wurzel. *Phytomorph.* **14**, 265-287.

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