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# The induced sector *Arabidopsis* apical embryonic fate map

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## SUMMARY

**Creation of an embryonic fate map may provide insight into the patterns of cell division and specification contributing to the apical region of the early *Arabidopsis* embryo. A fate map has been constructed by inducing genetic chimerism during the two-apical-cell stage of embryogenesis to determine if the orientation of the first anticlinal cell division correlates with later developmental axes. Chimeras were also used to map the relative locations of precursors of the cotyledon and leaf primordia. Genetic chimeras were induced in embryos doubly heterozygous for a heat shock regulated Cre recombinase and a constitutively expressed β-glucuronidase (GUS) gene flanked by the *loxP* binding sites for Cre. Individual cells in the two-apical-cell stage embryo responding to heat shock produce GUS-negative daughter cells. Mature plants grown from seed derived from treated embryos were scored for GUS-negative sector extent in the cotyledons and leaves. The GUS-negative daughters of apical cells had a strong tendency to contribute primarily to one cotyledon or the other and to physically adjacent true leaf margins. This result indicated that patterns of early cell division correlate with later axes of symmetry in the embryo and that these patterns partially limit the fates available for adoption by daughter cells. However, GUS-negative sectors were shared between all regions of the mature plant, suggesting that there is no strict fate restriction imposed on the daughters of the first apical cells.**

Key words: *Arabidopsis thaliana*, Fate map, Embryogenesis

## INTRODUCTION

Embryogenesis requires the activity of intrinsic and/or extrinsic positional fate cues to establish the developmental axes that guide the later formation of the body plan. Histological and tissue culture studies of early embryogenesis in angiosperm plants have resulted in the view that many plant embryos have a surprising mix of strictly regulative fate specification coupled with highly ordered patterns of cell division contributing to the organization of the body plan (Jurgens, 1992; Jurgens, 2001). However, the connection between the orientation of early cell divisions and later patterns of organ specification in embryos has been explored in only a few plant species (Christianson, 1986; Steffensen, 1968). In addition, few quantitative histological surveys of early embryogenesis in plants have been done, and those that have been performed have shown surprising diversity in both the relative timing and orientation of early cell divisions within some species (Christianson, 1986; Pollock and Jensen, 1964).

Extensive histological characterization of the embryogenesis of the dicotyledenous plant *Arabidopsis thaliana* has permitted some generalizations to be made regarding early patterns of cell division. The first zygotic cell division is usually asymmetric, creating an apical cell that eventually gives rise to the above ground portion of the shoot and a basal cell that contributes to the formation of the root meristem and the extraembryonic suspensor (Barton and Poethig, 1993; Mansfield and Briarty, 1991). Mutational analysis and hormone treatment experiments suggest that at this early stage the plant hormone auxin may act to help specify the unique fates of the apical and basal cell (Hamann et al., 1999). The apical and basal cells bear a number of signs indicating asymmetric fate specification, including different stain affinity in histological sections, and distinct patterns of cell division in daughter cells (Mansfield and Briarty, 1991; Mansfield et al., 1991).

The first division of the apical cell is anticlinal, occurring parallel to the long axis of the embryo. Following three mixed anticlinal and periclinal (perpendicular to the long embryonic axis) divisions in the daughters of the apical cells, a 16 cell embryo is produced displaying the first tissue differentiation with the establishment of the presumptive epidermis, the protoderm. Further divisions result in production of a spherical, globular stage embryo. The transition between globular and heart stage embryos results in a switch from radial symmetry to bilateral symmetry as the two cotyledon primordia emerge. As the cotyledons continue organogenesis, the hypocotyl begins to elongate to form the torpedo stage embryo; continuing cell elongation and division eventually causes the embryo to curve and fold over upon itself prior to seed set.

Following seed germination the bilaterally symmetric seedling, with two cotyledons located across the shoot apical meristem (SAM) from each other, emerges from the seed coat (Mansfield and Briarty, 1991; Medford et al., 1992).

Lineage-based approaches can be used to determine if the daughters of early embryonic cells show invariant or variable patterns of contribution to later-arising organs (Christianson, 1986; Dawe and Freeling, 1991; Steffensen, 1968; Woodrick et al., 2000). This report describes a fate mapping experiment with *Arabidopsis* following the lineage contributions of the daughter cells of the first two embryonic apical cells. A transgene system was used to create genetic chimeras with some cells containing the β-glucuronidase (GUS) marker gene and other cells in which the marker is removed. Excision of the marker occurs following the induction of Cre recombinase activity by cellular heat shock. A main goal of this study is to gain insight into whether early patterns of cell division predict the potential developmental contributions of apical daughter cells. If embryonic fate axes are specified prior to or during early divisions, it is likely that daughters of the first two apical cells will display stereotypic patterns of contribution to adult organogenesis. The fate map revealed that the physical orientation of the first anticlinal apical cell mitosis predicts the approximate location of the sagittal longitudinal plane (the plane of symmetry across which the two cotyledons reflect each other) in the post-germination seedling. However, lineage restrictions were not seen, suggesting that external cues, or a combination of internal and external cues, orient division planes during early apical mitoses.

## MATERIALS AND METHODS

### Transgenic plant lines and chimera induction

Creation of the Cre/*loxP*, GUS+|GUS–, sector generating system has been previously described (Sieburth et al., 1998). The two plant lines used in this study to generate genetic chimeras were HCN 3-3, carrying the Cre recombinase driven by the HSP 18.2 heat shock protein promoter (Takahashi and Komeda, 1989) and LoxAA13 carrying the 35S::GUS promoter/reporter fusion construct flanked by two *loxP* Cre binding sites. These lines have been used previously to generate clonal sectors lacking the GUS gene in early embryos and have been shown to provide generally strong GUS staining in vegetative tissue following germination (Sieburth et al., 1998). Plants doubly heterozygous for the HSP 18.2::Cre and *loxP*::35S::GUS::*loxP* constructs were created by performing genetic crosses between HCN3-3 and LoxAA13 homozygotes. All plants were raised in growth rooms maintained at 21°C with a 16/8 hour light/dark cycle. The $F_1$ embryos resulting from crosses were exposed to transient heat shocks by placing 2-day-old siliques (fertilized gynoecia) into water baths containing 39.5°C sterile, distilled water for 3 hours. Heat shock-treated plants were then returned to 21°C and allowed to set seed.

### Developmental staging of embryos

Expanding siliques were sectioned to establish the time after fertilization that two-apical-cell embryos were present. All embryos scored were $F_1$s derived from LoxAA13 × HCN3-3 crosses. Preliminary studies suggested that siliques collected 2 days after fertilization should contain embryos with two apical cells, thus siliques were collected during this period, fixed, stained, sectioned and examined using light microscopy as previously described (Torres-Ruiz and Jurgens, 1994). After determining that 2-day-old siliques contained embryos of the appropriate stage, the effect of heat shock at this point on later embryogenesis was examined. Siliques containing embryos allowed to develop for 6 days after heat shock and control siliques containing 8-day-old embryos that had never been heat shocked were fixed, sectioned and stained. Light microscopy was used to establish the relative stage of embryonic development in heat shocked and non-shocked seed pods.

### Identification of genetic chimeras

Staining to visualize the presence of GUS–|GUS+ genetic chimeras was performed on plants grown in axenic culture. Seed were surface sterilized, stratified at 4°C and grown as previously described (Woodrick et al., 2000). Plants with five to seven true leaves were harvested and submerged in a solution containing 1 mg/ml X-glucuronide (DDC Diagnostics Inc.) in 50 mM $NaPO_4$, 0.2% sodium dodecyl sulfate at pH 7.0 and then vacuum infiltrated for 20 minutes (Bossinger and Smyth, 1996). The vacuum was quickly released and infiltrated plants were incubated in partially vented tubes overnight at 37°C. Plants were removed from incubation and transferred through two rinses of 70% v/v ethanol before final storage in 70% ethanol. Chimeric and non-chimeric plants were counted and individual chimeras were transferred to vials for scoring of GUS-negative (GUS–) sector extent. The pattern of sector sharing between leaves and cotyledons was assayed by arranging chimeric plants on the surface of an agar pad (1% w/v agar in water) and individual videocaptures were taken of all plants.

### Developmental landmarks and sector assignment

In accordance with current practice in *Arabidopsis* fate mapping, all clonal plants identified were mapped onto model seedlings with the same phyllotaxy because handedness is thought to be randomly specified (Furner and Pumfrey, 1992; Irish and Sussex, 1992). For this study clockwise (rightward spiral) phyllotactic handedness was used as a standard, and the identity of cotyledons 1 and 2 (C1 and C2) and true leaves 1 through 4 (L1-L4) were assigned, based on phyllotactic position as previously described (Woodrick et al., 2000). The left and right side of each leaf is designated with the leaf placed tip up in relation to the viewer and with the adaxial (upper) leaf surface facing the viewer.

The extent of GUS– clonal sectors in mature plants was determined by analyzing each half cotyledon and each half leaf for both the cotyledons and for true leaves 1 through 4. Leaves were visually divided down the midline and then scored for the extent of GUS– sectors. Thus, any cotyledon or leaf was placed into one of four phenotypic classes; non-sectored, sector limited to the left margin, sector limited to the right margin or GUS– throughout. Sectors that crossed the midline of the leaf but failed to include all of the area of the leaf blade were scored as affecting only the half of the blade that was completely GUS–. However, the vast majority of GUS–|GUS+ sectored leaves had GUS– sectors that stopped at or about the midline of the leaf.

### Fate distance calculation and multidimensional scaling

Pair-wise developmental distances were calculated by assaying the frequency that GUS– sectors were shared between any two left and/or right marginal regions of the twelve marginal regions present in the cotyledons and first four true leaves. A total of 5799 sector sharing events between any two leaf margins were scored in 319 GUS–|GUS+ chimeras. Developmental distances were calculated using the following previously derived equation (Furner and Pumfrey, 1992).

$$D = 1 - \left[ \frac{2\times (\text{Number of seedlings with clones affecting both margins})}{\text{Total number of seedlings with clones in either margin}} \right] \times 100$$

Using this equation, different marginal regions that never shared a GUS– sector would produce a D value of 100 while those that always shared a GUS– sector would produce a D value of 0. Sector distribution within and between organs was scored by indicating if the

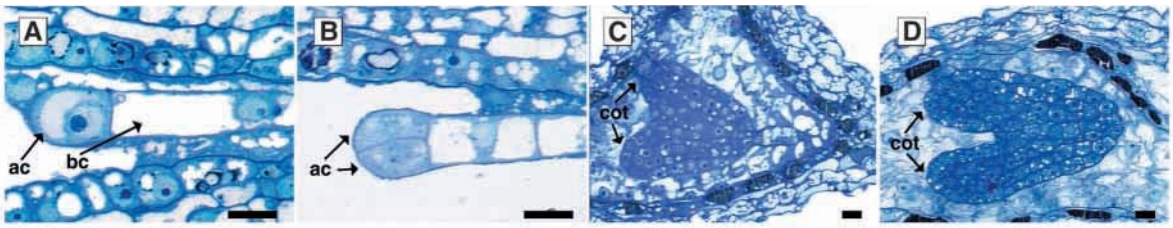


**Fig. 1.** Embryonic stage of induction of GUS–|GUS+ genetic chimeras. Whole ovules within gynoecia were sectioned and stained 2 days after fertilization (A,B) or 8 days after fertilization following no heat shock on day 2 (C) or a heat shock on day 2 (D). All embryos sectioned 2 days after fertilization had either a single apical and basal cell (A) or two apical cells separated by an anticlinal cell wall and two basal cells separated by a periclinal cell wall (B). Thus at the time of GUS– sector induction one or two apical cells were present in embryos. Eight days after fertilization both heat-shocked (D) and control (C) embryos were in the late heart to early torpedo stage of development, showing well developed cotyledon primordia and a root apex, suggesting that transient heat shock at the two-cell stage does not seriously disrupt later embryogenesis. ac, apical cell; bc, basal cell; cot, cotyledon primordia. Size bar: 10 μm.

left and/or right marginal regions were included in the GUS– sector. Thus, for example, a sector including the left side of cotyledon 1 is designated as a C1L sector while a sector including the right side of leaf 1 is designated as an L1R sector. A sector-sharing event between these two regions is designated as a C1L-L1R sharing event and is added to this event category. Data for all 319 chimeras were stored in a Microsoft Excel spreadsheet and spreadsheet cell equations and macros were used to calculate all pair-wise distances.

To identify total similarity between the sets of distances associated with each marginal region, metric multidimensional scaling incorporating prinicipal components analysis (MDS/PCA) was performed (Johnson and Wichern, 1992). A data matrix incorporating the number of sector events for each margin and the number of sector-sharing events between all possible margins was generated. This matrix was then used with the MDS/PCA procedure available in SAS, v8.1 (SAS Institute, Inc.). This analysis reduced the distance matrix into the two primary dimensions (principal components) containing the most variation based on all pair-wise distances. These primary dimensions were determined for each marginal region and plotted to scale. The two primary dimensions identify two axes that provide the nearest simulation (lowest stress model) of the total inter-item proximities seen in the 12 dimensional space occupied by the 12 marginal regions assayed in this study. The lowest stress model displays excellent monotonic goodness of fit with the original data set with stress values ranging from 1.3% to 2.3%.

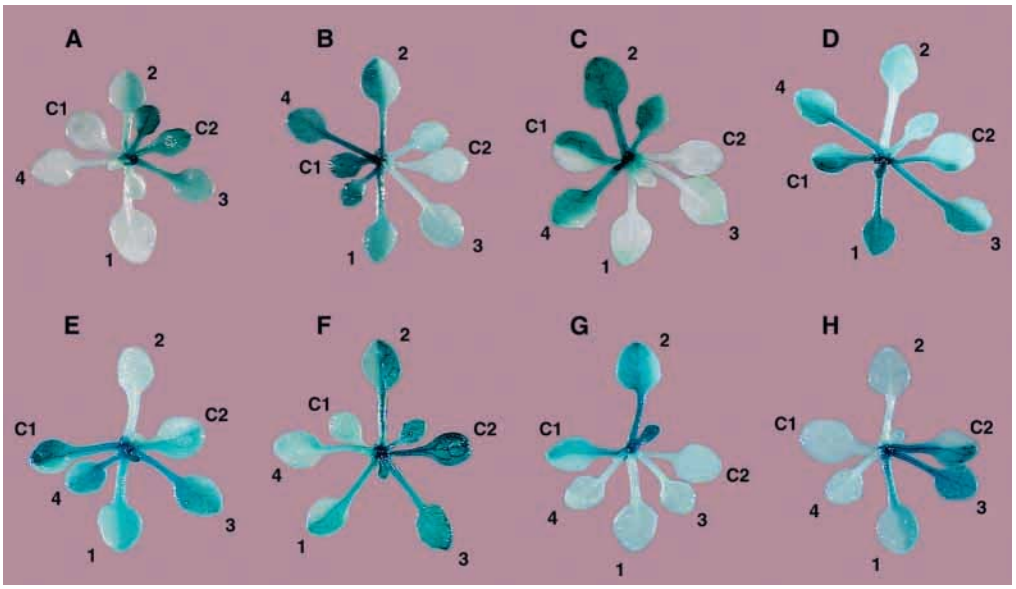


**Fig. 2.** Patterns of GUS– sector sharing seen in mature chimeric plants after sector induction at the two-apical-cell stage of embryogenesis. All plants are shown with clockwise phyllotaxy to permit easy visual comparison of sector sharing patterns. GUS– cell sectors are visualized as white tissue after clearing plants with ethanol while GUS+ cells retain blue coloration (A-H). Most GUS– sectors included all cells in one cotyledon and extended into physically adjacent organs (A,B) but many sectors also included one whole cotyledon and either the left or right margin of the other cotyledon (C). Rarer sectors extended either between cotyledons on the same side of the frontal longitudinal plane (D) or extended between the cotyledons but crossed the frontal plane (E). Most chimeras had sector boundaries that either separated the left and right margins of leaf 1 or leaf 2 (A,E,G-H) or separated the left and right margins of both leaf 1 and leaf 2 (B,F) which is reflected in the map as a tendency for GUS– sectors not to be shared between organs that lie across the sagittal longitudinal plane of the embryo. C1 and C2, cotyledon 1 and 2; 1-4, true leaves 1-4.

## RESULTS

### *Arabidopsis* embryogenesis and the effects of transient heat shock

The major goals of this study were to determine if the first apical cell division in the *Arabidopsis* embryo limits the potential patterns of daughter cell contribution to the cotyledons and first four true leaves and to create a fate map of apical organ specification. A heat-inducible Cre/*loxP* system was used to generate embryos chimeric for the presence of a 35S::GUS reporter transgene at the two-apical-cell stage, and the contribution of GUS– daughter cells to adult organs was determined. To establish both the time after fertilization that two-apical-cell stage embryos were present, and to determine if transient heat shocks altered normal embryogenesis, *Arabidopsis* embryos were assayed.

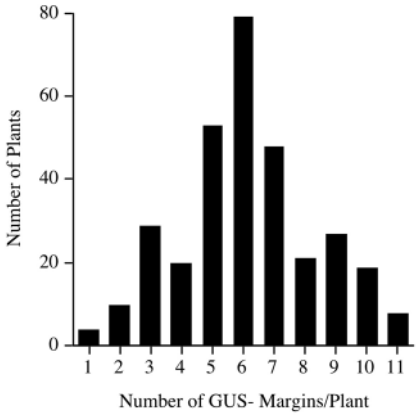


**Fig. 3.** Distribution of GUS– sector sizes among the population of 319 GUS–|GUS+ chimeras generated in this experiment. Chimeras were classified as having between 1 and 11 GUS– organ marginal regions on the basis of visual scoring. Local maxima in numbers of plants were seen at 3, 6 and 9 GUS– regions, roughly corresponding to plants that were 1/4, 1/2 or 3/4 GUS– in total organ area. Plants that are wholly GUS– (12 marginal regions GUS–) are not included because they are not apical chimeras and were not used for fate mapping.

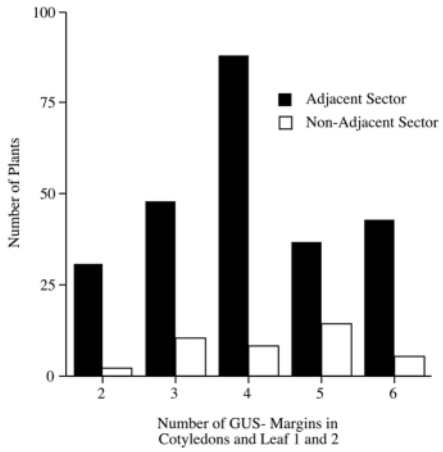


**Fig. 4.** Patterns of sector adjacency were scored for all chimeras used in this study. For this graph, only GUS– sectors in cotyledons and leaf 1 and 2 were scored for adjacency; these organs were selected because they are the oldest organs, with cotyledons being specified during embryonic development and leaf specification occurring soon after germination. The eight margins of these four organs were scored, however plants with only one, or those with seven or more, GUS– margins were omitted as their GUS– regions were wholly entire by default. The numbers of chimeric plants of a given sector size (two to six margins) are separated into chimeras with contiguous sectors and chimeras with non-contiguous sectors. Black bars show numbers of plants with the indicated number of GUS– margins whose sectors are wholly adjacent and white bars show the numbers of plants whose GUS– sectors show non-adjacency.

Microscopic analysis of sectioned ovules from fertilized, non-heat shocked, siliques (3 siliques sectioned, >20 embryos surveyed) indicated that all 2-day post-fertilization embryos fell into two developmental classes (Fig. 1A,B). All embryos seen were either at the two-cell stage, with one smaller apical cell and a larger basal cell, or at the four-cell stage with two apical and two basal cells.

To determine if early heat shock caused developmental defects during later stages of development, older heat-shocked and control embryos were subjected to histological examination. Eight-day-old embryos from three heat-shocked and non heat-shocked siliques were fixed, embedded, sectioned and stained. Histological analysis of the treated and control groups revealed that embryos ($n$=>20) from heat-shocked and non heat-shocked siliques were found to have progressed to the late-heart to early-torpedo stage of embryogenesis (Fig. 1C,D). This indicates that a short heat shock at the two-cell stage did not have a dramatic effect on the later progress of embryogenesis. No obvious lacunae in cell layers were seen suggesting that transient heat shock did not induce subsequent cell death, although immediate cell death and loss of a heat-shocked, GUS– cell at the two-cell stage can not be ruled out. However, if such an event occurred and led to a normal-appearing plant, it would not be chimeric and thus would not contribute to the analyzed dataset. Post-germination development was also compared between chimeric and control seedlings and no gross defects were detected (Fig. 2 and data not shown). Taken together, these data suggest that transient low level embryonic heat shock did not cause obvious developmental defects.

The identification of approximately equal numbers of one- and two-apical-cell stage embryos 2 days after fertilization suggests that half of the embryos responding to heat shock should give rise to all GUS– apical organs while half of the embryos should produce apical organ GUS–|GUS+ chimeras. To assay the relative proportions of wholly GUS–, chimeric, and non-responding embryos occurring after heat shock, a sample of 589 mature plants resulting from treated embryos was stained for GUS activity and sorted into appropriate phenotypic classes. In this population, 198 seedlings showed no apical GUS staining, although they generally displayed blue roots, 186 seedlings were GUS–|GUS+ apical chimeras and 205 seedlings were entirely GUS+. This indicates that approximately 65% of embryos responded to heat shock and of these responders 52% were wholly GUS– while 48% were chimeric in apical tissues. These results are in good accord with histological observation of approximately equal numbers of one- and two-apical-cell embryos in ovules at the time of heat shock and suggest that chimeras resulted from the generation of Cre recombinase in single cells during this developmental period. Plants wholly GUS– in apical tissues are not apical chimeras and were not used in subsequent analyses.

## GUS– sector size distribution

Plants derived from heat-shocked embryos were grown in axenic culture until four to six true leaves were produced and were then stained for GUS activity and assayed for GUS– sector size and extent. The extent of any GUS– sector was scored independently in six organs, the two cotyledons and the first four true leaves. For each of these organs, each half of the organ was also scored independently. Thus each plant was divided into 12 separate left and right marginal regions, and a GUS– sector on an apical chimera could have included between one and 11 organ margins. For the 319 chimeras utilized in this study, GUS– sectors were found that affected the entire range of organ margins. However, the most common

chimeras had between five and seven GUS– organ margins (Fig. 2 and Fig. 3). The majority of sectors were entire and adjacent, affecting marginal regions of physically adjacent organs, however clones were also seen in which sectors were non-adjacent and GUS– sectors were split by regions of GUS+ tissue. An analysis of the adjacency of sectors in the eight margins found on the cotyledons and first two true leaves indicated that plants with two to six GUS– margins consistently produced a minority of individuals with interrupted, non-adjacent GUS– sectoring (Fig. 4). In total approximately 14% of plants showed non-adjacent sectoring.

### The induced sector apical embryonic fate map

The extent of GUS– sector sharing between the cotyledons and/or true leaves was determined for a total of 319 GUS–|GUS+ chimeric seedlings. Developmental distances between all left and right leaf margins were calculated based on the frequency with which they shared GUS– sectors (Table 1). Developmental distances seen between the two cotyledons were among the highest distances seen in the embryo. All D values between C1 margins and C2 margins ranged from 60.3 to 71.2, indicating that sector sharing between the cotyledons occurred relatively rarely. Distances within cotyledons were almost half this distance, the C1L-C1R distance was 30.8 while the C2L-C2R distance was 27.3. This indicates that the two margins of a single cotyledon were more likely to be derived from a clonal, GUS– cell population, than any two margins between the cotyledons.

Because the inter-cotyledonary distances indicated that the distinct populations of daughter cells derived from two apical cells had a strong tendency to be incorporated into separate cotyledons, a similar bias might be expected for true leaf margins physically adjacent to the cotyledons. Chimeras with patterns showing GUS– sectors limited to either the right or the left cotyledon also tended to share sectors with adjacent true leaves (Fig. 2A,B and Table 1). Cotyledon 1 mapped closely to the adjacent regions of leaf 1 (L1R) and leaf 2 (L2L) while cotyledon 2 mapped closely to adjacent true leaf regions L1L and L2R. However, given the close mapping of the adjacent regions of L1R to C1 and L1L to C2, both cotyledons mapped quite far away from the L1 margin that is not adjacent to them (Table 1). L1L is 58.0 D away from C1L and 65.2 D away from C1R while L1R is 63.6 D away from C2R and 67.4 D away from C2L. Leaf 2 also displayed close mapping to adjacent cotyledon margins and large distances between L2 margins and non-adjacent cotyledon margins.

In a separate analysis, multidimensional scaling was performed on a dissimilarity (distance) matrix generated from the sector sharing data to produce a graphical representation of patterns of covariation in distances between marginal regions. This analysis tested the internal consistency of the data set by comparing all pair-wise distances between marginal regions against each other and, based on how commonly different sector patterns were shared, generating new distance values. Multidimensional scaling identified two major axes (principal components) that together account for 93.7% of the variation in the pair-wise distances (Fig. 5). The major dimension (Dimension 1), accounting for 81.4% of variation in the data, separates the margins into two groups associated with each cotyledon. The minor dimension (Dimension 2), accounting for 12.3% of variation in the data, split each cotyledon-associated group of margins in two. Thus, four clusters of margins with highly covarying sets of distances were identified, Cluster 1 including C1L, L1R and L4L, Cluster 2 including C1R, L2L and L4R, Cluster 3 including C2L and L2R and Cluster 4 including C2R, L1L and L3L and R. One other dimension accounting for less than 3% of the variation in the data suggested a pattern of association involving the cotyledon margins (not shown). All other dimensions each accounted for less than 1% of total variation.

**Table 1. Pairwise development distances between all organ marginal regions**

| | C1L | C1R | C2L | C2R | L1L | L1R | L2L | L2R | L3L | L3R | L4L |
|---|---|---|---|---|---|---|---|---|---|---|---|
| C1R | 30.8 | | | | | | | | | | |
| C2L | 71.2 | 60.3 | | | | | | | | | |
| C2R | 69.9 | 70.9 | 27.3 | | | | | | | | |
| L1L | 58.0 | 65.2 | 39.0 | 22.8 | | | | | | | |
| L1R | 17.3 | 38.9 | 67.4 | 63.6 | 48.3 | | | | | | |
| L2L | 38.2 | 14.6 | 56.2 | 65.6 | 62.8 | 40.8 | | | | | |
| L2R | 59.7 | 50.6 | 19.3 | 37.2 | 45.1 | 56.9 | 40.4 | | | | |
| L3L | 66.8 | 67.2 | 22.1 | 15.1 | 26.3 | 60.0 | 60.8 | 30.6 | | | |
| L3R | 68.5 | 69.5 | 28.7 | 12.8 | 16.4 | 59.7 | 64.2 | 36.9 | 11.8 | | |
| L4L | 19.3 | 29.6 | 70.0 | 71.2 | 57.8 | 16.5 | 34.8 | 58.4 | 69.8 | 66.5 | |
| L4R | 31.8 | 19.2 | 63.9 | 70.1 | 62.2 | 33.8 | 14.1 | 47.5 | 66.2 | 67.4 | 24.3 |

Developmental distances represent the percentage of time GUS– clones are not shared between any two organ margins. Red numbers indicate distances less than 50 D, purple numbers represent the developmental distance across organs. To determine developmental distance between two regions identify one region on the bottom axis and the other on the side axis. Their intersection in the table is the developmental distance between the regions.

## DISCUSSION

### Timing of GUS– sector induction

All fate mapping and lineage analyses depending on genetic systems for chimera induction suffer from uncertainties concerning the timing and position of marked sector induction (Sturtevant, 1927). Using a heat-shock regulated Cre/*loxP* system to induce GUS–|GUS+ genetic chimerism also presents possible difficulties. Even though heat shock was induced in embryos with one or two apical cells (Fig. 1) it is possible that the time of action of the heat shock response or Cre recombinase activity was delayed or extended by one or more cell divisions. Fully GUS– apical chimeras were recovered at about a 2% higher frequency than GUS–|GUS+ apical chimeras, suggesting that some two-cell embryos may have undergone two independent heat shock events. However, because these embryos produced seedlings with fully GUS– aerial organs, they were not used in subsequent analyses. At the opposite extreme, 43 chimeras had sectors extending through 3 or fewer margins (Fig. 3), suggesting that these chimeras were induced in one cell in a four apical cell embryo, indicating either a delay in recombinational excision of GUS or a delay in heat-shock activation of Cre. In addition,

approximately 14% of the chimeras used in this study had non-adjacent GUS– sectoring (GUS– regions interrupted by GUS+ tissue) (Fig. 2E, and Fig. 4). Two possible causes of this are embryos with two independent sector-generating events in two of four apical cells, or embryos in which a cell division resulted in one GUS– daughter cell becoming repositioned between two GUS+ cells. This type of sector interdigitation via cell division has been well documented in a number of plant chimeras (Poethig and Sussex, 1985; Steeves and Sussex, 1989; Szymkowiak and Sussex, 1992). Patterns of sharing and covariation in adjacent and non-adjacent clones are similar (data not shown) indicating that non-adjacent chimeras do not contribute confounding data to distance and covariation calculations. The totality of clone patterns seen in this study support the conclusion that the vast majority of chimeras used were induced by single events during the two- to four-apical-cell stage.

## Embryonic apical cell division and a fate axis

The regular pattern of relative cell placement and the highly stereotyped patterns of localized gene expression seen in globular stage and older *Arabidopsis* embryos has led to the suggestion that these embryos are partitioned into distinct organ and tissue anlage' (for a review, see Jurgens, 2001). Although an embryo plan emerges during the globular stage, it is unclear whether this map is dependent on specific cell lineages and patterns of division established earlier in development, or instead is a result of pattern formation arising de novo during the globular stage (Long and Barton, 1998). If early cells establish lineages that bias the organization of the globular stage fate map, it is likely that genetic chimeras induced during the initiation of the apical region of the embryo would produce daughters that have constrained patterns of contribution to the mature embryo and seedling.

Upon completion of embryogenesis and the germination of seed, the *Arabidopsis* seedling displays simple orthogonal double mirror symmetry in the longitudinal axis, with one plane of symmetry running down the center of the two cotyledons and the other plane falling between the cotyledons. The first plane has been referred to by other authors as the frontal longitudinal plane and the second as the sagittal longitudinal plane (Long and Barton, 1998). If patterns of early cell division correlate strongly with later globular stage developmental axis specification, it is likely that the plane of cell division producing the first apical cells would tend to establish either the frontal or the sagittal plane of the embryo. Alternatively, if globular stage pattern formation is independent of earlier cell lineages, no correlation should be seen between the establishment of cell lineages at the first apical cell division and later specification of developmental axes.

Using a data set of 319 chimeras generated by sector induction events applied during the one- or two-apical-cell stage, patterns of GUS– sector sharing were surveyed and developmental distances were calculated between marginal regions of mature organs. The longest developmental distances in this study are seen when the organ margins under consideration lie across the sagittal longitudinal plane from each other. Comparing pair-wise developmental distances, any distance in which the sagittal longitudinal plane is crossed is large (Table 1) and the distances between the cotyledons were always among the largest seen in the seedling. This pattern indicates that the first apical cell division plane generates two populations of daughter cells with a strong tendency to contribute to portions of the globular embryo lying on one side or the other of the sagittal longitudinal plane. However, GUS– clone sharing is seen between all marginal regions of the embryo (Fig. 2). Thus, any influence of the orientation of the first plane of apical cell division on the later partitioning of the embryo must not depend on strict lineage-dependent cell fate specification. This result supports the model that globular stage pattern formation depends on the de novo specification of organ and meristem anlagen. The first cell division appears to orient the first two apical cells such that their daughters tend to be incorporated into either cotyledon one or two and the physically adjacent regions of leaves 1 through 4. Thus, the orientation of the first apical cell division appears to predict the future sagittal plane and to partially constrain fate choices available to daughter cells. However, the relative positional identity of the first apical cells, or the position of the first, anticlinal apical cell wall, is unlikely to be strictly specified by mosaic determinants because sector sharing between organs lying across the sagittal plane from each other does occur.

This conclusion is supported by another fate mapping experiment previously performed in cotton. In contrast to the *Arabidopsis* embryo, which acquires a dermatogen after eight apical cells are formed, the cotton embryo acquires a dermatogen late in development, when the embryo proper has

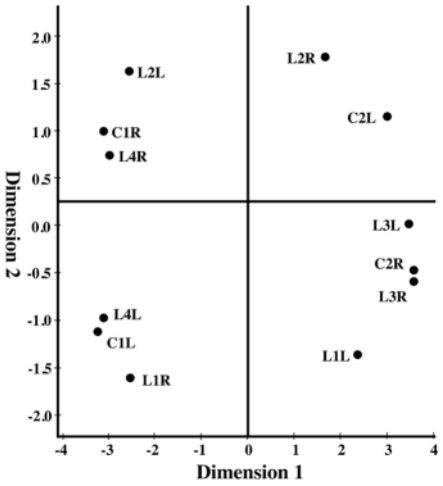


**Fig. 5.** Results of multidimensional scaling analysis. This plot was prepared by clustering marginal regions based on the first two primary dimensions identified in the pairwise distance matrix. The first dimension accounts for the greatest axis of dissimilarity in the data, and clusters margins from the two cotyledons and their close-mapping partners away from each other across the sagittal longitudinal plane. The second dimension accounts for the next largest degree of dissimilarity in the data, and clusters the left and right margins of the cotyledons and leaf 4 away from each other across the frontal longitudinal plane. Both dimensions account for 93.7% of the variation in the original data matrix. Taken together these data highlight the fact that the daughters of the first apical cell division are not randomly apportioned to all organ margins with uniform frequency. C1L, C1R, C2L and C2R indicate the left and right margin, respectively, of cotyledon 1 and 2. Other labels similarly indicate the left and right marginal regions of true leaves 1-4.

between 50 and 100 cells in total (Pollock and Jensen, 1964). Thus, a considerable period of cell division intervenes between the first apical cell division and the formation of a globular stage embryo. Semigametic cotton lines produce haplo-diploid and haploid-haploid two-apical-cell chimeras because of a failure of micro and megagametophyte pronuclear fusion in the zygote (Turcotte and Feaster, 1973). Visible markers associated with this chimerism have been used to construct a cotyledonary fate map (Christianson, 1986). Interestingly, in this experiment 86% of the recovered chimera sectors included 50% or more of the total organ area, and the largest class of chimeras displayed sectors covering 75% of total organ area. This indicates that most marked cell sectors outgrew their unmarked neighbors, contributing the bulk of cells to the embryo. However the resulting cotyledons produced by these embryos were normally shaped, sized and positioned. The cotton embryonic fate map suggests that dicot embryonic pattern formation events applied to cell populations primarily derived from one apical cell can result in normal embryogenesis. Thus, in both cotton and *Arabidopsis*, early patterns of cell division may constrain the relative position of daughter cells, influencing their ultimate fate specification, but their cell division lineage is unlikely to contribute directly to that fate determination.

### The Organogenic fate map

The inter-marginal developmental distance matrix generated in this study plots the relative size and position of the anlagen for the cotyledons and true leaves 1 through 4. Not surprisingly, the specification events for the cotyledons mapped extremely distantly from each other (Table 1), probably due to their relative positions at distant points on the embryo and due to the fact that the apical cell daughters tend to contribute to half of the embryo. Each cotyledon appears to be much more likely to be completely derived from the daughters of one of the first two apical cells than to recruit cells derived from the daughters of both cells. Thus, developmental distances between these organs reflect both their true, relative positions and biases imposed by patterns and relative orientations of early cell divisions.

The sagittal plane increases the developmental distances between any margins that lie across the plane from each other, even if those margins occupy the same organ, as seen in the relatively large developmental distances seen between the left and right margins of leaf 1 (L1) and leaf 2 (L2) (Table 1). The left and right sides of L1 and L2 map on average twice as far apart as do these margins from adjacent margins of other true leaves and the cotyledons. Since the sagittal plane marks the midline of L1 and L2, it is interesting to note that the large intermarginal distances seen across these organs indicate that they have a strong tendency to draw founder cells from the daughters of both apical cells seen at the two-apical-cell stage. Leaves 3 and 4 arise on either side of the sagittal plane and show close mapping to adjacent regions of older organs, while also mapping distantly from any organ mapping to the other side of the sagittal plane.

An analysis of the covariation of developmental distances generated in this study also highlights the impact of the sagittal plane (Fig. 5). Multidimensional scaling and principal components (MDS/PCA) analysis assessed the covariation of all distances on a margin by margin basis. Thus this analysis demonstrated, for instance, that pair-wise developmental distances including margin C1L and any other margin covary most closely with pair-wise distances including margin L4L and any other margin. These analyses are useful because they address a key assumption of fate maps, that they produce probabilistic representations of the real relative physical positions of fate specification events. Thus, if two fate specification events occur in immediately adjacent regions of the embryo, they should first map closely together, and secondly map similar distances away from the cohort of all other mapped fate positions. The results of MDS analysis shown in the plot are broadly similar to the pattern of fate distances seen in the developmental distance table (Table 1 and Fig. 5), and highlight the strong tendency for organ margins on each side of the sagittal plane to have covarying distances. These analyses show that C1, L1R, L2L and L4 distances covary as do distances including C2, L1L, L2R and L3 marginal regions. On average, GUS– sector sharing events within these groups are between 25 and 50% more likely than are sharing events between these groups.

The patterns of covariation in the distance data also may indicate that there is a closer total similarity between the distances generated between L3 and L4 margins and all other margins and distances that include the cotyledonary margins adjacent to L3 and L4. The covariation map suggests that L3 and L4 primordia may be physically more closely associated with adjacent cotyledons than indicated by the developmental distance data alone. Thus, MDS analysis of data derived from biological fate mapping experiments provides an additional approach to identify patterns of association between anlage, through visual inspection of a graphical representation of all transformed distances, than might be noted when fate distances alone are considered.

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