

A fate map of the *Arabidopsis* embryonic shoot apical meristem

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Summary

We have mapped the fate of cells in the *Arabidopsis* embryonic shoot apical meristem by irradiating seed and scoring the resulting clonally derived sectors. 176 white, yellow, pale green or variegated sectors were identified and scored for their position and extent in the resulting plants. Most sectors were confined to a fraction of a leaf, and only occasionally extended into the inflorescence. Sectors that extended into the inflorescence were larger, and usually encompassed about a third to a half of the inflorescence circumference. We also find that axillary buds in *Arabidopsis* are clonally related to the subtending

leaf. Sections through the dry seed embryo indicate that the embryonic shoot apical meristem contains approximately 110 cells in the three meristematic layers prior to the formation of the first two leaf primordia. The histological analysis of cell number in the shoot apical meristem, in combination with the sector analysis have been used to construct a map of the probable fate of cells in the embryonic shoot apical meristem.

Key words: *Arabidopsis*, clonal analysis, meristem, fate map, genetic mosaics.

Introduction

The cells of the shoot apical meristem proliferate throughout a plant's lifetime to give rise to the differentiated tissues of the shoot. We would like to understand how the cells in the meristem acquire information about their position and then divide and differentiate accordingly to form both vegetative and reproductive organs. Two schools of thought have provided different models to explain how these patterns of development come about. Buvat (1955) proposed that specific cells in the summit of the shoot apical meristem were set aside early in development as a mitotically quiescent population, which would provide the initial cells solely for the reproductive structures. An alternative theory was proposed by Wardlaw (1957), who suggested that cells in the summit of the shoot apex divide to contribute to all of the organs and tissues of the shoot. Histological examination of mitotic figures in the shoot apex in a number of angiosperm species indicates that cell divisions do occur throughout the meristem, but cannot provide information on the ultimate fates of these cells (see Steeves and Sussex, 1989, for review).

A more detailed understanding of the fate of meristematic cells comes from clonal analysis. By constructing polyploid single cell layer chimeras, Satina et al. (1940) first demonstrated that there are three cell layers that give rise to reproducible portions of the plant. The outer, or L1, cells give rise to the epidermal structures, while the inner L2 and L3 layers give rise to the subepidermal mesophyll, cortex and vascular tissues of the plant. Similarly, the contribution of a single meristematic cell to the differentiated tissues can be ascer-

tained by genetically marking a single cell in the shoot apical meristem and examining the resulting clone of marked cells. Meristematic cells have been marked by inducing or uncovering a mutation using ionizing radiation or chemical mutagens (for reviews, see Stewart, 1978; Poethig, 1987; Irish 1991). These clonal analysis experiments have demonstrated that meristematic cells do appear to proliferate in a predictable manner to form the differentiated tissues of the plant; however, these experiments also demonstrate that cell fate is not dependent on cell lineage, but rather the differentiated fate of a cell depends on its position (Stewart, 1978).

Such clonal analyses have been carried out extensively in maize. By monitoring the contributions of marked cell lineages to the differentiated tissues of the plant body, a fate map of the embryonic shoot apical meristem of maize has been constructed (Steffensen, 1968; Coe and Neuffer, 1978; Johri and Coe, 1983; McDaniel and Poethig, 1988). Although the sectors recovered in these experiments show variability in size and extent, a general pattern of sector distribution could be identified. These studies indicate that meristematic cells in the maize embryo proliferate in a regular manner, such that different regions of the meristem give rise to successive, overlapping regions of the mature corn plant. Based on a high proportion of induced sectors restricted to the tassel, Coe and Neuffer (1978) and Johri and Coe (1983) suggested that the maize tassel arises from a discrete population of cells in the embryonic meristem. However, later work (McDaniel and Poethig, 1988; Poethig et al., 1990) suggested that this apparent lineage restriction reflects the dynamics of meristem growth, and that if vegetative

growth is prolonged then meristem cells that would otherwise only give rise to the tassel can also contribute to vegetative tissues as well. These data indicate that there are no separate, lineage restricted apical initials in the maize meristem.

Clonal analysis in *Arabidopsis* has focused on the number of meristem cells that contribute to the germ line. By assaying the frequency of recovery of different mutations in the progeny of irradiated plants, a number of researchers have estimated the number of meristematic cells that give rise to the reproductive tissues of the inflorescence, or the 'genetically effective cell number' (GECN). The GECN in the dry seed shoot apical meristem of *Arabidopsis* has been estimated to be about 2 or 3 cells (Muller, 1965; Li and Redei, 1969; Grinikh et al., 1974; Grinikh and Shevchenko, 1976). However, little attention has been paid to the contributions of meristem cells to the somatic tissues of the *Arabidopsis* plant.

Arabidopsis is an indeterminate plant, and under long-day conditions produces about eight vegetative rosette leaves and then bolts to form the inflorescence. By a combined histological and clonal analysis of growth dynamics in the meristem, we have been able to estimate the probable number of cells, and their position, in the dry seed shoot apical meristem that give rise to each of the leaf primordia and the inflorescence in *Arabidopsis*. Our studies also demonstrate that lineages that give rise to vegetative portions of the plant can also contribute to the reproductive tissues. However, the width and extent of sectors in our study suggests that proliferation of meristematic cells in *Arabidopsis* follows a different pattern than that demonstrated for other plant species.

Materials and methods

Seedlings were prepared for histological analysis and scanning electron microscopy by fixing in FAA (3.7% formaldehyde, 50% ethanol, 5% acetic acid) for one hour and then dehydrating in an ethanol series. Dehydrated material was prepared for sectioning by passing the material into tertiary butyl alcohol and embedding in Tissue Prep 2 (Fisher). 6µm sections were cut and affixed to polylysine-coated slides, dried and deparaffinized. Slides were stained in toluidine blue, dehydrated and mounted in Permount (Sigma), and observed with bright-field optics using a Leitz Aristoplan microscope. Dehydrated material was prepared for electron microscopy as previously described (Irish and Sussex, 1990). Dry seeds were similarly prepared, except that fixation was accomplished by autoclaving seed in FAA for 30 minutes and then processing as above.

Arabidopsis seeds (ecotype Landsberg *erecta*) were mutagenized at 125 rad / min in a Co⁶⁰ gamma ray source maintained by Yale University. Seeds were planted immediately after irradiation in flats containing 12:3:1 Vermiculite: soil: sand, grown at 20-22°C, and watered daily. Plants were grown under a 16 hour day / 8 hour night photoperiod, with a combination of fluorescent and incandescent lighting (175 µmole m⁻² sec⁻¹). Plants that contained sectors were transplanted into individual 3 inch pots for further analysis.

Results

Anatomy of the dry seed shoot apical meristem and early stages of vegetative development

The dry seed stage shoot apical meristem of *Arabidopsis* is flat and consists of about 110 small, densely staining cells, with about 36-38 cells in each of the histogenic layers. The number of cells in the meristem was estimated by counting cells in both serial longitudinal and transverse sections from individual fixed meristems. Figure 1A and B show representative sections. By 48 hours after imbibition, the meristem has enlarged in a plane perpendicular to the cotyledons (Figs 1, 2) to create a ridge. The ends of this ridge gives rise to the first two leaf primordia, which become apparent by 96 hours.

The first two leaf primordia appear similar in size and shape at 96 hours, and relatively large stipules are present at the base of both leaf primordia (Fig. 2). By 192 hours, however, it is clear that one of the first two leaves is larger and somewhat more advanced developmentally, as evidenced by the appearance of trichome primordia. The advanced development of one of the first two leaf primordia is probably a stochastic process. At this stage, the third leaf primordium is apparent, and is somewhat in advance of the fourth leaf primordium (Figs 1H, 2G). Phyllotaxy in *Arabidopsis* is helical and is randomly clockwise or counterclockwise. The establishment of the first leaf and the position of the third leaf is sufficient to establish a clockwise or counterclockwise phyllotaxy. The phyllotactic pattern becomes obvious at later stages (Fig. 2). The meristem remains flat until the floral transition at about 200 hours (Vaughn, 1955; Miksche and Brown, 1965).

Induction and distribution of sectors in the shoot apical meristem

In order to estimate the level at which irradiation-induced damage occurs, we exposed *Arabidopsis* seed to different doses of irradiation (Fig. 3). Seed germinated at the normal rate with up to 120 krad of gamma rays. However, irradiation above 60 krad was detrimental to the subsequent rate of growth, as well as to viability. In subsequent experiments, sectors were induced at 40 krad, which was estimated to be the highest dose at which minimal irradiation damage occurred in the meristem.

Approximately 10,000 seed were planted immediately after 40 krad of gamma-irradiation, and sectors in the resulting population were recovered at a frequency of about 2% (172 sectorized plants). Four plants carried two genotypically distinct sectors, as assessed by their different color and appearance in different leaves. These sectors were scored as independent events. 168 white, yellow, or pale green, and 8 variegated clonally derived sectors were recovered and scored for their size and extent. Since the L1-derived epidermis is essentially colorless, the sectors that we observed must be due to clonally inherited defects in the subepidermal tissues. Very few L3 sectors were recovered, and only L2 sectors were scored in these experiments. In addition, several sectors were recovered in which the size of the leaf containing the sector was markedly reduced. These sectors were not included in the analysis. Representative sectors are illustrated in Fig. 4, and a tabulation of sector types recovered is presented in Fig. 5.

The variety in sector phenotype presumably results from different types of mutagenic events. Chlorophyll-deficient sectors induced in a wild-type background have been docu-

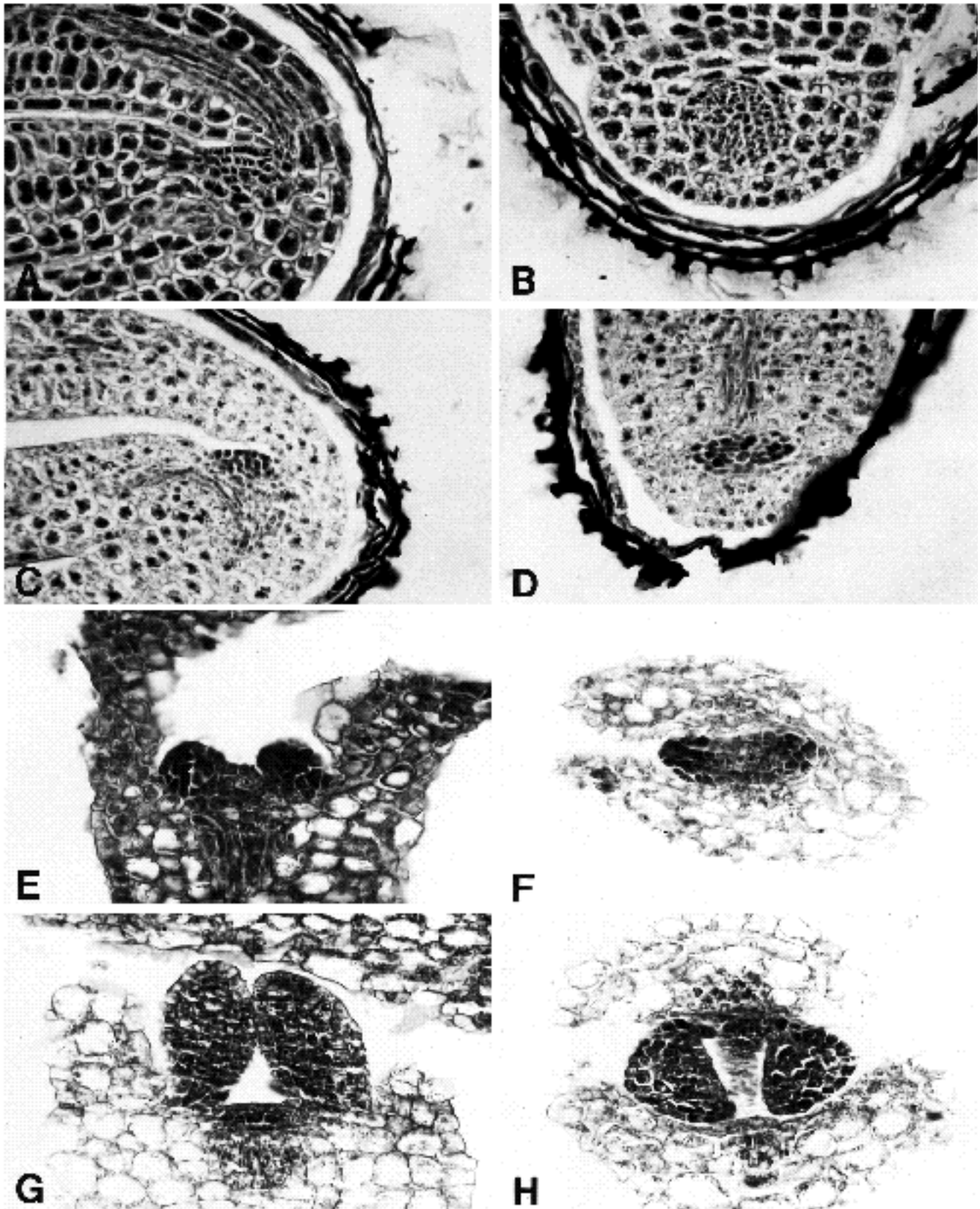


Fig. 1. Sections through *Arabidopsis* shoot apical meristems at different stages of development. (A, C, E, G) Longitudinal sections; (B, D, F, H) transverse sections through the meristem. (A, B) Tissue harvested at the dry seed stage; (C, D) 48 hours, (E, F) 96 hours, and (G, H) 144 hours after imbibition; note the appearance of the third leaf primordium in (H).

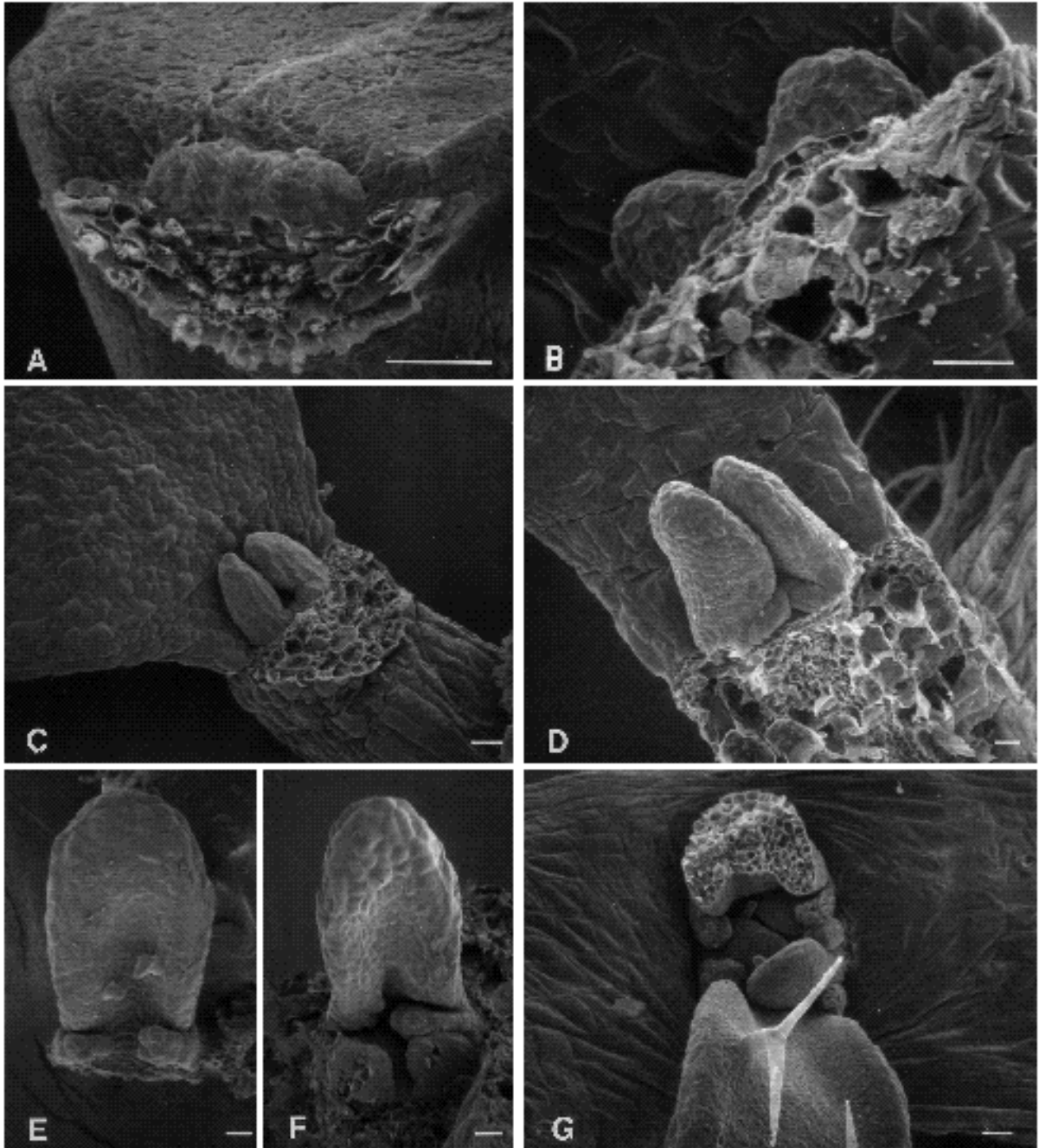


Fig. 2. Scanning electron micrographs of *Arabidopsis* shoot apices at different stages of development. In A-D, one cotyledon has been removed. (A) Meristem 48 hours after imbibition; (B) 96 hours; (C) 144 hours with stipules beginning to be apparent; (D) 192 hours, with prominent stipules formed at the base of both leaves. (E, F) First two leaves from an individual 192 hour seedling, note trichome primordia initiation in E. (G) 240 hour apex showing helical phyllotaxy. Leaf 2 has been removed. Scale bar = 20 microns.

mented in other systems (Jegla and Sussex, 1989). In maize, ionizing radiation results in chromosome breakage and subsequent loss of chromosomal fragments, resulting in aneuploid cells (Stein and Steffensen, 1959). Loss of linked markers on the same chromosome arm following irradiation has

also been observed in *Arabidopsis*, and probably reflects loss of a chromosomal fragment (Hirono and Redei, 1965). Aneuploidy for several genomic regions in *Arabidopsis* can be detected by an altered color phenotype (Lee-Chen and Steinitz-Sears, 1967; Koornneef and Van der Veen, 1983), so

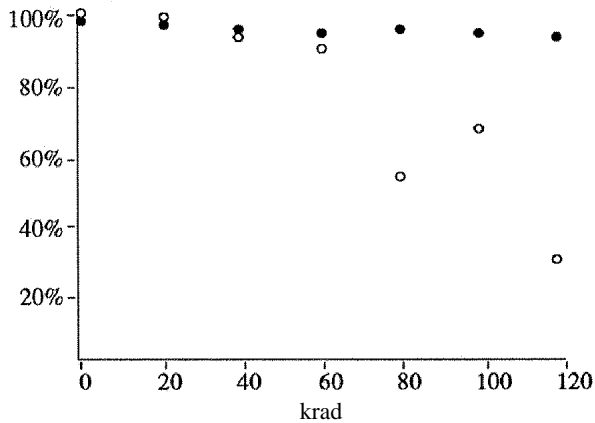


Fig. 3. Dose response of dry seeds to different levels of ionizing radiation. ● percent of seeds planted surviving to germination. ○ percent of seeds planted surviving to flowering. Flowering was measured as the percent of plants that had bolted at least 1 cm by 28 days after planting.

we suspect that the loss of part or all of a chromosome in a meristematic cell, resulting in an aneuploid cell lineage with an associated color phenotype, is the cause of these phenotypically detectable sectors. The eight variegated sectors that we obtained may be due to chloroplastic mutations. However, a mutant chloroplast would have to replicate and sort out during subsequent cell divisions until the entire population of chloroplasts in a cell carried the defect. Since all eight variegated sectors were detectable at the base of the leaf, and dicots in general have about 10 to 20 plastids per meristematic cell (Possingham, 1980), it is unlikely that a sorting out process could account for the early appearance of these variegated sectors.

In general, sectors only populated part of a single leaf (Fig. 5A). About 10% of the sectorized plants had multileaf sectors (Fig. 5B), or sectors that passed into the inflorescence. Multileaf sectors reflect the proximity of the initials for the affected leaves; this estimate of distance based on the frequency with which organs are contained in the same as opposed to different clonal sectors has been used to construct fate maps in other systems (Sturtevant, 1929). Of the seven sectors that extended into the inflorescence, three were initiated in the rosette, demonstrating that a single marked meristem cell can give rise to both vegetative and floral tissue.

We also examined the relatedness of axillary buds to leaves. We observed twelve sectors in the axillary inflorescences arising from nodes within the rosette. In eleven plants, the sector was observed both in the subtending leaf and in the axillary bud. In one case, a sector spanning the marginal quarter of leaf 5 continued into the axillary inflores-

cence of leaf 7. In a separate experiment, plants with vegetative sectors were repeatedly decapitated to induce axillary bud outgrowth from the sectorized leaves (E. Bahn and I.M.S., unpublished). Of 98 single-leaf sectors, 34 had sectorized tissue in the axillary bud. In the other 64 plants, no further sectoring was observed.

Estimate of primordium cell number

The frequency of sector occurrence in a given leaf reflects the number of cells in the dry seed stage shoot apical meristem that gives rise to that leaf. The observed frequencies were higher for leaves one and two, and decreased with increasing leaf number (Table 1). Multiplying the frequency of sector occurrence by the estimated number of L2 cells (based on histological observations) at the time of irradiation results in an estimate of the number of L2 cells in the dry seed shoot apical meristem that give rise to each leaf (Table 1). This estimation of primordium cell number depends on the assumption that all cells in the shoot apical meristem are equally sensitive to irradiation. A major factor influencing radiation sensitivity is the rate of cell division. Since irradiations were carried out on dry seed, uniform sensitivity is a reasonable assumption.

The calculation of the apparent cell number (ACN) is another method that has been widely employed to estimate the number of cells in a primordium (for review, see Poethig, 1987). The ACN equals the reciprocal of the fractional width that a sector occupies. The accuracy of the ACN as an estimate of actual primordium cell number depends on a variety of factors, including relatively uniform cell division rates and cell expansion throughout the leaf. In addition, the calculation of the ACN can vary by a factor of two depending on whether chromosomal damage occurred in the affected cell at the two-strand or four-strand stage of DNA replication (Poethig, 1987). Because of this inherent variability, we have presented the range of ACN that we calculate for the width of each leaf (Table 2). Because almost all of our sectors extended from the base to the tip of the leaf, the ACN for the length of all leaves is one, suggesting that each leaf is derived from a single subepidermal rank of cells tangential to the shoot apical meristem (Fig. 5). Furthermore, visual examination of some of these plants indicates that the sector spans both upper and lower mesophyll, which also suggests that only one rank of meristematic cells contributes to the entire subepidermal layer of the leaf.

Many of our sectors terminate at the midvein; we suspect that this represents a difference between the growth and expansion of cells in the lamina and in the midvein rather than a cell lineage restriction. Poethig and Sussex (1985a,b) noted a similar discontinuity of sectors at the boundaries of

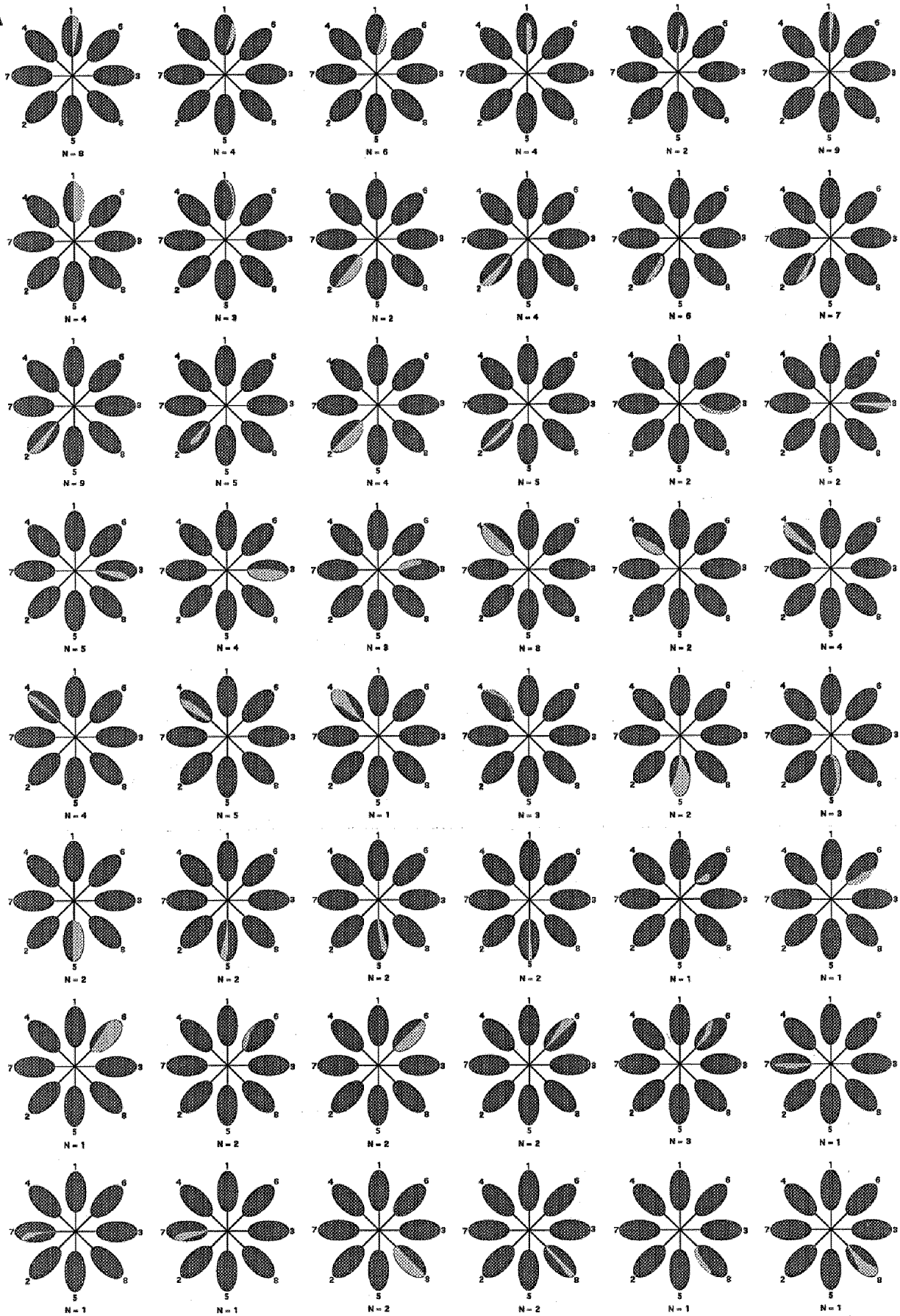
Table 1. Frequency of sector occurrence in each leaf and in the inflorescence

	Leaf number								
	1	2	3	4	5	6	7	8	INF
Number of sectors ^a	39	45	21	27	19	17	3	12	7
Frequency	21%	24%	11%	14%	10%	9%	2%	6%	4%
Approximate number of L2 cells for each leaf primordium ^b	8-9	8-9	4-5	4-5	3-4	3-4	1	1-2	1-2

^a, multileaf sections were scored as occurring in each of the leaves affected.

^b, calculated by multiplying the number of L2 cells at the time of irradiation by the frequency of sectors obtained.

5A



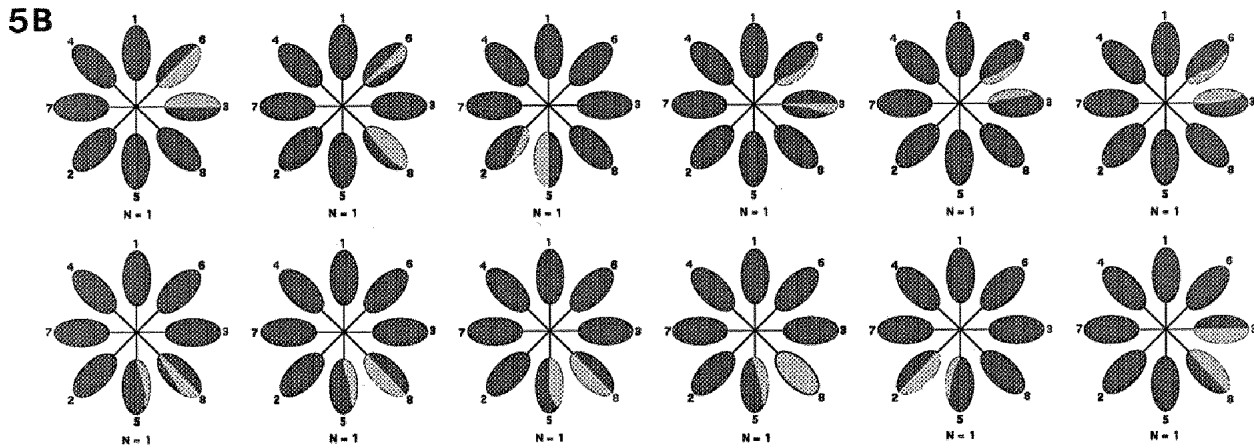


Fig. 5. Summary of sector types. The size and extent of vegetative sectors are illustrated schematically, with the eight rosette leaves numbered. All sectored plants have been normalized to a counterclockwise phyllotaxy. The number of sectors occupying a similar extent of a given leaf is indicated below each diagram. (A) Summary of sectors occupying only a single leaf. (B) Summary of multileaf sectors.

Table 2. Summary of the ACN for each leaf

	Leaf number							
	1	2	3	4	5	6	7	8
Range of ACN	2-16	2-16	2-8	2-8	2-8	2-8	2-8	2-8
Average ACN	5.4	5.3	4.4	4.4	4.5	4.5	4.5	4.6

lateral veins in tobacco leaves. They showed that, in young tobacco leaves, vascular tissue occupies about half of the leaf primordium, but occupies significantly less than half of the mature leaf, suggesting that the high incidence of clonal boundaries at lateral veins reflects differential cell growth.

A fate map of the embryonic shoot apical meristem

The histological observations of the cell numbers in the meristem indicate that a single layer of the embryonic shoot apical meristem contains about 36 to 38 cells. Based on a hexagonal packing array, we can construct a fate map for the L2 layer of the meristem that contains 37 cells (Fig. 6). By combining the results of the frequency analysis, the calculation of the ACN for each leaf, and the data from multileaf sectors, which indicates which primordia are adjacent, we can assign the most likely fates of L2 cells within the dry seed shoot apical meristem. The ACN analysis suggests that each leaf is derived from one rank of cells within the subepidermal layer of the shoot apical meristem, and can be derived from a somewhat variable number of cells within that rank. We have based the estimated width of the leaf primordia on the results obtained from the frequency analysis, since this measure should not be subject to the artifacts inherent in the ACN calculation. However, the calculated ACN for each leaf does agree with the estimated number of cells in each primordium derived from the frequency analysis. In addition, the data from the multileaf sectors confirmed the placement of adjacent primordia on the map. We should point out that we can only score a sector if it falls into leaf tissue; our fate map does not reflect the possibility of a significant proportion of meristematic cells giving rise to internodal tissues. However, we think this possibility is unlikely since *Arabidopsis* is a rosette

plant with essentially no internodal elongation during vegetative growth.

Although we have not calculated the map of fates of cells within the L1 or the L3 layer, we suspect that cells in these layers behave similarly to the L2 cells. The fate map we have illustrated represents the most likely fate of L2 cells within different regions of the meristem, but is not meant to imply that these fates are lineage-restricted.

Discussion

Each sector that we have generated represents a clone of cells derived from a single mutagenic event that took place at the dry seed stage. We have estimated the number and location of L2 cells in the meristem that give rise to a particular primordium by assessing the size and distribution of sectors using several different methods. Since many sectors occupied similar, but not identical, regions of the plant, meristem cells are not fated to give rise to a specific portion of the plant body, but do appear to have fairly regular and reproducible destinies. Using the sector data, we have constructed a map of the probable fates of cells in the dry seed shoot apical meristem. Although these types of maps are generally referred to as 'fate maps', we emphasize that this map represents the probability that a given meristem cell will contribute to a particular organ. We would like to suggest the use of the term 'probability map' to distinguish this mode of development from that of lineage-restricted development typified by *Caenorhabditis elegans* (Sulston and Horvitz, 1977).

Most of the gamma-ray-induced sectors that we observed were restricted to a wedge-shaped region of a single leaf. This is in contrast to sectors induced with high levels of ethylmethane sulfonate (EMS; I.M.S., unpublished), which in

general are wider and extend through a greater number of nodes. We suspect that the EMS mutagenesis protocols used result in a high level of cell death in the shoot apical meristem, since germination in these experiments is only about 75% of the unmutagenized control (I.M.S., unpublished). This high degree of cell death would lead to enhanced sector size, since the original population of surviving meristematic cells is smaller. Increase in the size of the mutant sector, and therefore a decrease in the calculated ACN, occurs at high doses of mutagens (Muller, 1965; Mednic and Usmanov, 1985). In pilot experiments, low doses of EMS result in minimal lethality, and produce a distribution of sectors comparable to those seen using gamma-rays (I.M.S., unpublished). By limiting the dose of gamma-rays to a level at which non-specific radiation damage is negligible, the sectors produced probably accurately reflect the cell division patterns produced by unmutagenized plants.

The probability map that we have generated for the *Arabidopsis* dry seed shoot apical meristem differs in certain respects from fate maps generated for other plant species. We find that, in general, sectors extend from the base to the tip of a given leaf, suggesting that one rank of cells in the meristem is recruited into forming the subepidermal tissues in a single node. In maize and sunflower, most sectors induced at the dry seed stage extend through a number of nodes (Steffensen, 1968; Coe and Neuffer, 1978; McDaniel and Poethig, 1988; Jegla and Sussex, 1989). In addition, the sectors obtained in these studies showed a great deal of variability in both sector width and extent, indicating that meristem cells can give rise to a variable amount of the plant body. In maize, these results have led to the construction of a dry seed stage fate map that is composed of overlapping domains of increasingly more apical levels of the meristem, containing several ranks of cells, each giving rise to a number of nodes on the plant (Coe and Neuffer, 1978; McDaniel and Poethig, 1988). Most of the sectors that we have recovered do not show significant variability in extent in that they fall discretely into one leaf; however, we do see occasional multileaf sectors, indicating that although in the majority of cases a single rank of cells gives rise to only one node, we do have evidence that a rank of cells can give rise to a greater extent of the plant body.

Poethig et al. (1986) have suggested that the most frequent termination of sectors in a region of the maize plant corresponds to the transition of growth from one ring of meristem cells to the next inner ring. However, a discontinuity in sector termini has not been observed in other studies (McDaniel and Poethig, 1988; Jegla and Sussex, 1989). The apparent periodicity observed in sector termini in some maize studies and in our observations of *Arabidopsis* may not reflect the actual transition from one rank of cells to the next in the meristem. Instead, these observations may reflect a discontinuity in the cell growth and division patterns that give rise to different organ primordia. Similar to the probable cause of frequent sector termination at the midvein, a lack of uniform cell growth at the boundary between node and internode could result in a higher frequency of sector terminations.

In *Arabidopsis*, axillary buds are clonally related to the subtending leaf. We obtained 12 sectors in axillary buds, 11 of which were also sectoried in the subtending leaf. This is in contrast to the case in maize, where axillary buds (the ear shoots) are clonally related to the internode above, rather

than to the subtending leaf (Johri and Coe, 1982; McDaniel and Poethig, 1988). Histological studies of axillary bud development in several angiosperms suggest that cells in the axils of leaf primordia remain small and undifferentiated, as detached meristems (Garrison, 1955; Sussex, 1955). These studies suggest that different populations of meristematic cells give rise to the axillary buds and to the subtending leaves. Although these primordia are histologically distinct, other studies have demonstrated an inductive relationship between leaf and axillary bud development (Snow and Snow, 1942). The clonal relationship of axillary buds and the subtending leaf in *Arabidopsis* suggests that, in this species, the axillary bud may be derived from the young leaf primordium, rather than from a separate population of detached meristematic cells.

Sectors induced on later leaves have a greater probability of populating two or more nodes, indicating that cells in the outermost rank of the meristem are more restricted in their fate. These results suggest that cells appear to acquire a fate as they enter the flanks of the meristem. A similar observation in semigametic cotton, in which clonal sectors were induced early in embryogenesis, suggested that cells that will give rise to the first two leaf primordia are set aside during the organization of the shoot apex in cotton, and so have restricted fates (Christianson, 1986). Both of these observations imply that, despite the pluripotency of plant cells, cells in the meristem acquire information that limits their subsequent differentiation. These results are important in light of the wealth of data now available on the patterns of gene expression in the meristem. A sophisticated understanding of the dynamics of cell growth and division in the meristem will provide us with the tools necessary to interpret these molecular prepatterns and relate them to the events occurring during plant morphogenesis.

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Fig. 4. Sample sectors. (A) Typical vegetative sector; (B) sector that passes into the inflorescence and completely populates one cauline leaf and its axillary inflorescence. Magnification (A) 2×; (B) 10×.

Fig. 6. *Arabidopsis* probability map. (A) Schematic illustration of the L2 layer of the *Arabidopsis* dry seed shoot apical meristem, represented by 37 hexagonal cells. The regions of the meristem that are most likely to give rise to a certain region of vegetative tissue are color coded to match the illustration of the mature rosette in B. The black region in the center of the map has a high probability of giving rise to inflorescence tissues. (B) Color-coded schematic illustration of the mature rosette, with leaf numbers indicated.