The Plant Cell, Vol. 4, 631–643, June 1992

Normal and Abnormal Development in the Arabidopsis Vegetative Shoot Apex

June I. Medford,[a,1] Friedrich J. Behringer,[a] Joseph D. Callos,[a] and Kenneth A. Feldmann[b]

[a] Department of Biology, The Pennsylvania State University, University Park, Pennsylvania 16802
[b] Department of Plant Sciences, University of Arizona, Tucson, Arizona 85721

Vegetative development in the Arabidopsis shoot apex follows both sequential and repetitive steps. Early in development, the young vegetative meristem is flat and has a rectangular shape with bilateral symmetry. The first pair of leaf primordia is radially symmetrical and is initiated on opposite sides of the meristem. As development proceeds, the meristem changes first to a bilaterally symmetrical trapezoid and then to a radially symmetrical dome. Vegetative development from the domed meristem continues as leaves are initiated in a repetitive manner. Abnormal development of the vegetative shoot apex is described for a number of mutants. The mutants we describe fall into at least three classes: (1) lesions in the shoot apex that do not show an apparent alteration in the shoot apical meristem, (2) lesions in the apical meristem that also (directly or indirectly) alter leaf primordia, and (3) lesions in the apical meristem that alter meristem size and leaf number but not leaf morphology. These mutations provide tools both to genetically analyze vegetative development of the shoot apex and to learn how vegetative development influences floral development.

INTRODUCTION

Arabidopsis has become a focus for molecular genetic approaches to understand complex processes such as development. An elegant model for floral development has recently been advanced based on data from molecular and genetic studies on Arabidopsis and Antirrhinum (Coen and Meyerowitz, 1991). These studies show that many aspects of floral form are regulated in the inflorescence and floral apical meristems. The vegetative apical meristem gives rise to a distinct shoot form, so key processes regulating vegetative shoot development should also be localized in the apical meristem. In addition, the genetic controls that regulate vegetative development may also affect genes functioning in floral development.

Development from the vegetative shoot apex is both similar to and different from development from the floral shoot apex. Normal floral development follows a sequence in which a series of organs are initiated (sepals, petals, stamens, and carpels). Vegetative development also initiates a sequence of lateral organs as it proceeds from juvenile to adult phase (Poethig, 1990). In spite of similar sequential processes, most of the mutants described to date that regulate floral development are not thought to have a role in vegetative development (e.g., Yanofsky et al., 1990; Bowman et al., 1991; Schultz and Haughn, 1991; Shannon and Meeks-Wagner, 1991). In addition to initiating sequential units, vegetative development is also repetitive. Repetition occurs as the vegetative meristem continuously initiates organs. This repetitive developmental pattern is not found in normal floral development.

Mutants affecting the Arabidopsis vegetative shoot apical meristem have not been described. A number of factors contribute to the difficulty in isolating mutants affecting vegetative meristem development. First, the vegetative meristem is deeply buried in more mature tissues, making its direct observation difficult. Second, whereas the apical meristem is involved in the inception, and perhaps early development of organs, most development occurs after the organs are initiated. Therefore, a visual alteration in the morphology of the vegetative shoot apex may or may not be the result of a lesion in the vegetative meristem. Third, although cell lineage studies in cotton indicate a separate lineage for the cotyledons and apical meristem (Christianson, 1986), no data are yet available for Arabidopsis. Therefore, mutations disrupting cotyledon formation may or may not include vegetative meristem mutations. Finally, mutations disrupting the vegetative meristem may result in embryos or seedling lethality.

Two previous works provide a starting point for studying Arabidopsis vegetative development. Vaughn (1955) analyzed the transition from vegetative to reproductive development using an unspecified ecotype. In a study more specific to vegetative development, Miksche and Brown (1965) briefly described cellular changes in the shoot apex and meristem using in vitro grown plants of the Estland ecotype. Although these studies have provided useful information, many other processes not described in these studies are taking place. For example, there is no information on the meristem prior to 4

[1] To whom correspondence should be addressed.

days after germination. Furthermore, Miksche and Brown (1965) describe the initiation of leaves one through four with an opposite phyllotaxy, but observation of an Arabidopsis rosette shows that later leaves are initiated with a spiral phyllotaxy. No information is available concerning changes in the Arabidopsis apex that accompany the altered phyllotaxy.

Some recent studies on plant shoot apices have used the terms "shoot apex" and "apical meristem" with broad definitions or interchangeably (Ursin et al., 1991; Yamamoto et al., 1991). However, based on decades of careful studies on meristems (Wardlaw, 1957), Cutter (1965) unequivocally defined the "apical meristem" as the part of the shoot lying distal to the youngest leaf primordia, whereas "shoot apex" was defined as comprising the apical meristem and young leaf primordia. As elaborated by Wardlaw (1957), the apical meristem comprises the "embryonic initial cells" as well as the region in which "the inception of growth centers takes place." These regions are more commonly known today as the central zone and the peripheral zone, respectively. The shoot apex comprises the apical meristem as well as the organogenic region where "the outgrowth of leaf primordia takes place," the subapical region where there is "considerable widening" of the shoot and enlargement of the primordia, and the region of maturation. These definitions of shoot apex and apical meristem are in current usage (Steeves and Sussex, 1989) and allow the description of detailed changes in vegetative development. We have used these definitions throughout our description of the Arabidopsis vegetative shoot apex.

Within the apical meristem, distinctive regions or zones are found (Cutter, 1965; Besnard-Wibaut, 1977; Esau, 1977). Although cells in the zones do not have fixed fates, they usually follow predictable patterns. Cells at the sides of the apical meristem, or peripheral zone, typically produce lateral appendages such as leaves. Cells at the summit or central zone are a source of cells to other regions and function as nonpermanent initials (Steeves and Sussex, 1989). Finally, cells at the base of the meristem make up the rib meristem or rib zone, which contributes to formation of the stem. We have made a detailed study of changes in the Arabidopsis vegetative meristem and used this information as a basis for describing meristem mutants. The mutants were initially identified as disrupting the vegetative shoot apex. However, we show that not all disruptions in the shoot apex are evident as disruptions in the apical meristem. Such information and mutants will be useful in constructing a model for vegetative development and analyzing how aspects of vegetative development do or do not affect floral development.

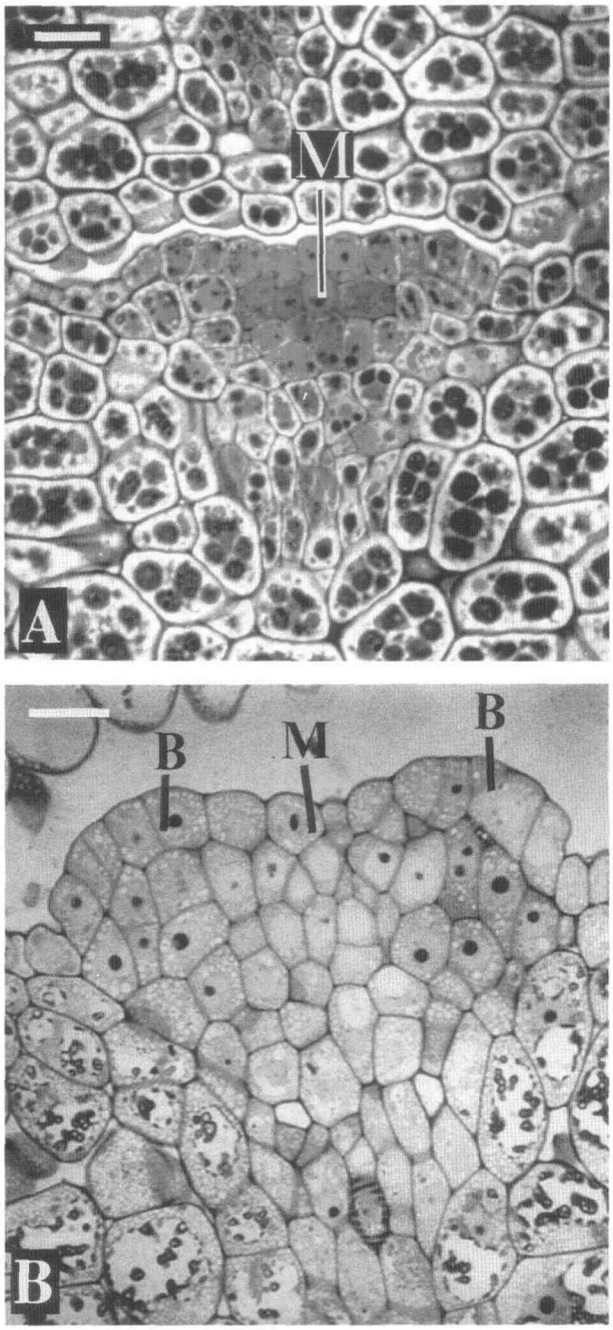

Figure 1. Sections through Young Shoot Apical Meristems.

(A) Section through apical meristem in a mature seed imbibed for 12 hr. Cells of the apical meristem have large nuclei that occupy most of the cell.

(B) Section through apical meristem in a 2-day-old seedling. Cells in the meristem's periphery show prominent nucleoli, whereas nuclei in the central zone cells stained lightly. Throughout the meristem an intricate network of cytoplasmic strands and vacuoles is evident.

B, bulges; M, shoot apical meristem. Bars = 10 μm.

RESULTS

Description of the Young Shoot Apical Meristem

A detailed description of early Arabidopsis embryogenesis has been published (Mansfield and Briarty, 1991). Figure 1A shows

a section through a mature seed imbibed for 12 hr at 4°C. The apical meristem is flat with dimensions of approximately 35 × 55 μm. This apical meristem is among the smallest in the dicots and comparable in size to that of the Arabidopsis globular embryo (Mansfield and Briarty, 1991). The cells of the meristem are cuboidal to rectangular in longitudinal view, measuring 4 to 10 μm in width with a nucleus (diameter of 2.6 to 4.5 μm) that often fills much of the cell. The depth of the apical meristem varies from four cell layers in the center to two layers at the margin, and the entire apical meristem contains approximately 60 to 80 cells. Two bulges on the meristem surface may result from the initiation of the first and second leaves late in embryogenesis. However, because there are no apparent periclinal cell divisions in the second cell layer (L2), it is difficult to definitively describe the slight bulges as developing primordia. Figure 1B shows that 2 days later initiation of the first leaf primordia pair is definitive, but meristem zones are still not distinct. One primordium is slightly ahead of the other, suggesting that they are not initiated simultaneously. In the meristem, only the outermost cell layer is distinctive. However, the cell number in the meristem has increased to approximately 110 to 130 cells. This increase is most noticeable in a plane perpendicular to the cotyledons and results in a rectangular meristem.

Figure 2 shows the shoot apex viewed at 4 days using a scanning electron microscope (SEM). The meristem is flat and rectangular in shape. The meristem has a distinct bilateral symmetry, whereas the primordia have a radial symmetry (Figure 2A). Stipules have developed on both sides of each primordium's base. The first external evidence of differentiation in a primordium is a developing trichome at the distal most end (Figure 2B). Differentiation of the distal most trichome is precocious relative to other trichomes. The primordia have a diameter of 36 μm with approximately 66 cells in each primordium at this point (cell number calculated as described in Methods). Because the vegetative shoot meristem initiates units known as phytomers, cells for axillary buds must also be initiated at this point. However, there is no distinct appearance of axillary precursors for leaves one and two in either sections and SEMs of the early shoot apex.

Further development in the shoot apex is apparent in both the meristem and leaf primordia. Figure 3A shows that the previously radially symmetrical primordia (cf. Figure 2) have flattened and are assuming a dorsiventral form. In addition, the adaxial trichome at the distal most portion of one primordium has further differentiated. Even though the developing leaf has a dorsiventral symmetry at this point, the blade and petiole are not yet distinct. Figure 3B shows that the meristem is still flattened, but instead of the rectangular shape seen earlier (Figure 2), it now has a trapezoidal shape. The trapezoidal shape is associated with both changes in cell size and in cell number. In cross-section, the width of the meristem varies from four cells at the narrow end to six cells at the wide end. This difference in the meristem width may reflect the emergence of the third leaf primordium. Primordium three is initiated at the widest end of the meristem, often in a position closer to one of the first two primordia rather than equidistant between the first two primordia. Stipules from leaves one and two overtop the new primordium. Figure 3C shows a longitudinal section through the shoot apex, further suggesting that primordium three is initiated before primordium four. However, periclinal divisions in the second cell layer indicate that the inception of primordium four is not long afterward. In addition, Figure 3C shows that the meristem zonation has become more distinct. Cells of the central zone can be distinguished by their large centrally located nuclei, whereas cells in the peripheral zone have smaller nuclei and more defined vacuoles. Cell number in the meristem has continued to increase so that the trapezoidal meristem contains approximately 170 cells.

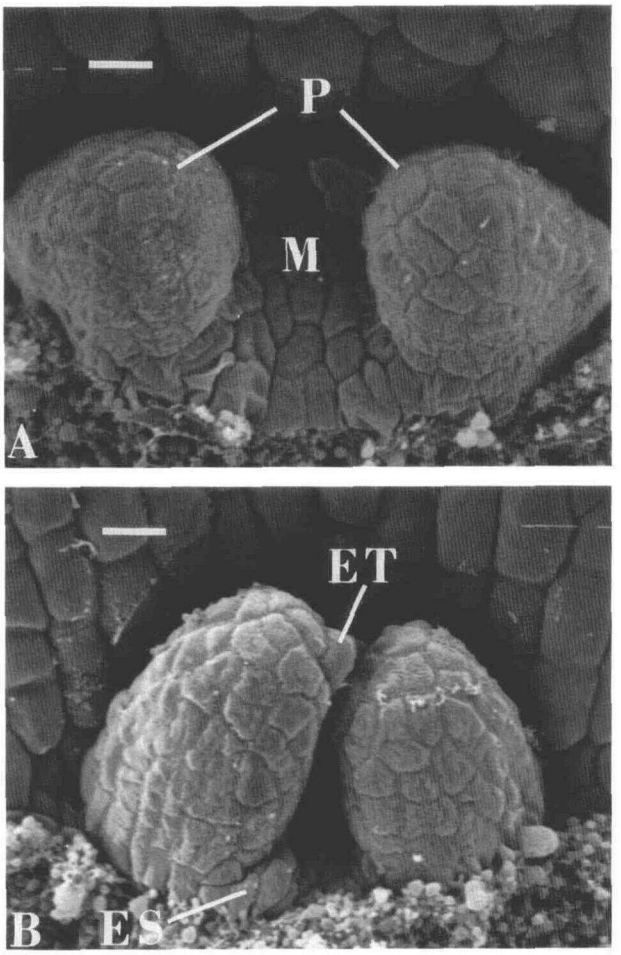

Figure 2. Leaf Primordia of Developing Shoot Apex at 4 Days.

(A) Near vertical view showing that the first leaf primordia pair has a radial symmetry and is formed on opposite sides of the rectangular meristem.

(B) Side view showing that the primordia with stipules and trichomes begin to form.

ES, emerging stipule; ET, emerging trichome; M, meristem; P, primordia. Bars = 10 μm.

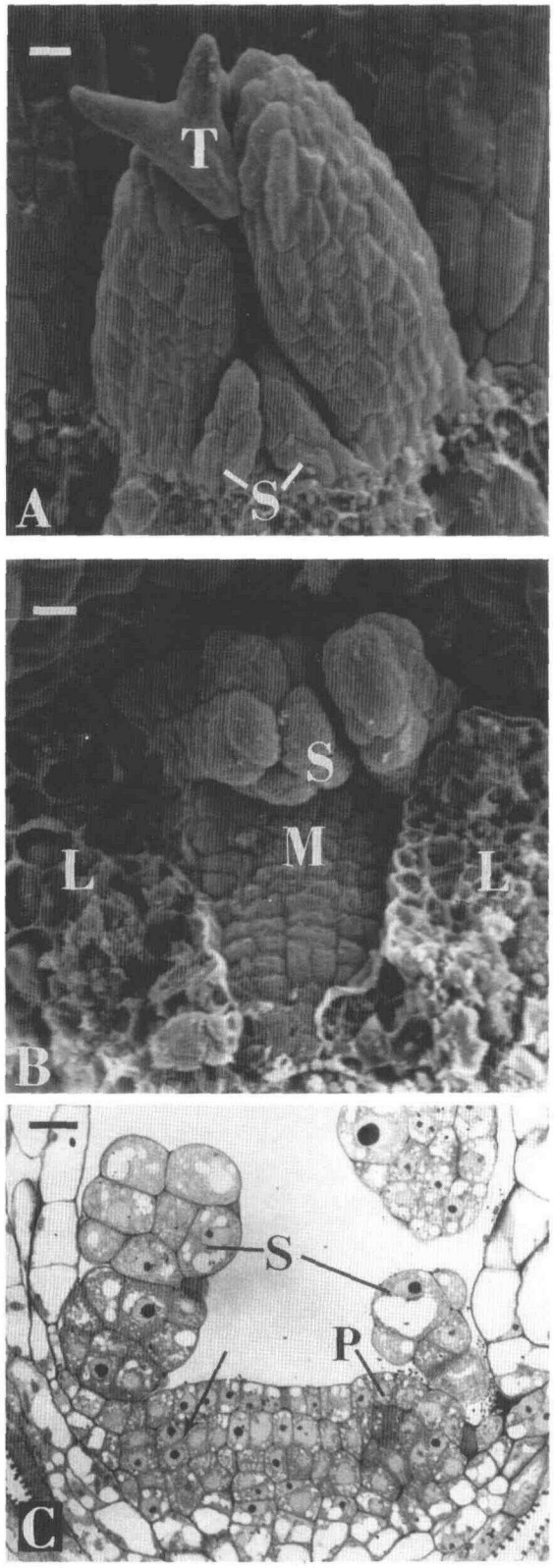

Figure 3. Differentiation in the Shoot Apex at 5 to 6 Days.

Figure 4A shows that the third and fourth leaf primordia are initiated on opposite sides of the meristem at an angle approaching 180°. However, the initiation angle and the angle of emergence often vary. Figure 4B shows that leaves three and four are emerging at an angle much less than 180°. The emergence angle was variable from plant to plant. However, a change in the emergence angle was not observed in leaves one and two, and the alteration may be in anticipation of the switch in phyllotaxy that follows the initiation of leaf four (see below). Differences in initiation angle and emergent angle have been reported in other species (Clowes, 1961). Figure 4B also shows that the stipules for leaves one and two are becoming necrotic.

Description of the Mature Shoot Apical Meristem

The meristem in the early shoot apex (Figure 2) is a flattened rectangle with bilateral symmetry. Figures 5A and 5B show that as the plant ages the meristem expands and becomes dome shaped. When viewed with SEM, the terminal portion of the shoot apex appears triangular (Figure 5A), in contrast to the dome-shaped meristem seen in longitudinal section (Figure 5B). This indicates that the triangular terminal portion of the shoot apex (Figure 5A) represents a meristem with developing primordia at three points. The meristem increases in width from 35 to approximately 70 μm and now contains approximately 450 cells. However, average cell size in the domed apical meristem does not change significantly. In the domed meristem, the peripheral and central zones are more distinct. In addition, cell division in two cell layers (L1 and L2), rather than one cell layer, becomes restricted to an anticlinal plane. The apical meristem remains as a radially symmetrical dome through inflorescence and floral states (Smyth et al., 1990).

Heteroblastic Leaves Correspond to an Altered Meristem

Leaves initiated from the juvenile meristem have a shape distinctive from those of the adult meristem. Early in Arabidopsis

---

(A) The first leaf primordia pair becomes dorsiventral. The stipules and distal most trichome are fully differentiated.

(B) Near vertical view showing a trapezoid-shaped meristem partially covered by club-shaped stipules of the first leaf pair. The bases of leaves one and two are on either side of the meristem.

(C) Near median section through the meristem. The meristem is still flat, and the third leaf primordium can be seen as a slight bulge. The arrow points to a periclinal division marking the initiation of the fourth primordium on the opposite side of the meristem from the third primordium.

L, leaves; M, meristem; P, primordia; S, stipules; T, trichome. Bars = 10 μm.

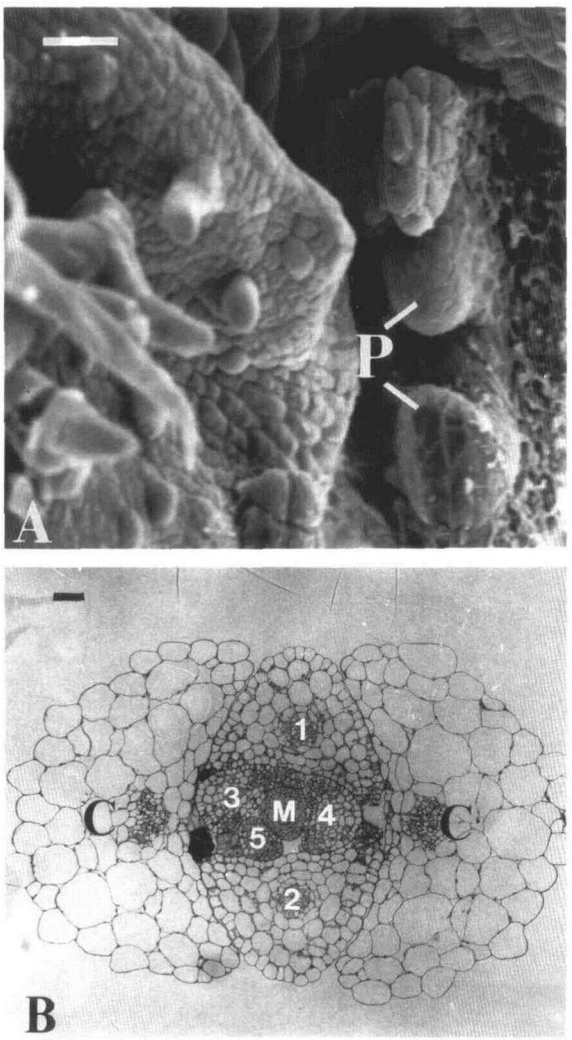

Figure 4. Comparison between Initiation Angles and Emergence Angles of Third and Fourth Leaf Primordia.

(A) SEM showing that the third and fourth primordia are initiated opposite one another.

(B) Cross-section through 7-day-old shoot apex showing the third and fourth leaf primordia at angles greater than 180° as the fifth primordium develops. Stipules from leaves one and two are dark staining and necrotic.

M, meristem; C, cotyledon; P, primordia. Leaves are numbered from oldest (1) to youngest (5). Distinction between first and second primordia is arbitrary. Bars = 25 μm.

development, the leaves are round and entire, whereas older adult leaves are spatulate and serrate. Our observations corroborate those of a previous study on heterophyllic changes in Arabidopsis (Röbbelen, 1957). Also in agreement with the previous study, we observed a correlation between increased meristem size and more complex leaf shapes. Arabidopsis plants do not become capable of reproductive development prior to formation of the fifth rosette leaf. This, along with the change in leaf form, suggests that the bilaterally symmetrical meristem represents the juvenile form of the apical meristem, whereas the domed radially symmetrical meristem represents the adult form.

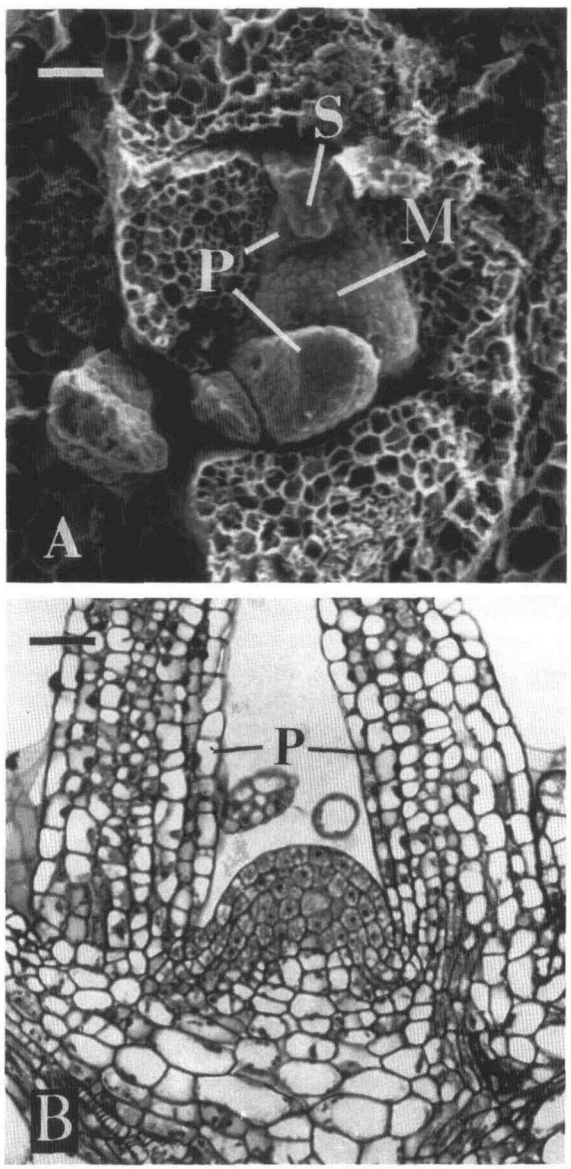

Figure 5. Radially Symmetrical Adult Meristem.

(A) SEM of mature vegetative meristem. Leaf primordia develop at the corners of the meristem and are overtopped by stipules.

(B) Median longitudinal section through 7-day-old shoot apex showing that the meristem is dome shaped. The central zone, peripheral zone, and rib zone are more distinctive than seen earlier (cf. Figure 3C).

M, meristem; P, primordia; S, stipule. Bars = 25 μm.

Mutations Disrupting Vegetative Organ Development

The above work provides a basis for describing mutations that disrupt vegetative shoot development. To isolate mutations affecting vegetative development, we screened a 10- to 16-day-old population of Arabidopsis plants for alterations in the shoot apex. These plants had been subjected to mutagenesis by T-DNA insertion (Feldmann, 1991). All mutations reported here segregate as recessive lesions in nuclear genes. Figure 6 shows an example of a mutation disrupting vegetative development. The *curly* (*crl*) mutation causes the cotyledons and leaves to expand abnormally and become hyponastic (Figure 6A). In Figure 6B, abnormal expansion in the developing leaf primordia can be seen (cf. Figures 6B with 5B), but there is no obvious alteration in the apical meristem. However, this does not rule out an alteration in the apical meristem on a cellular level. The Crl abnormality is found in all organs formed from the apical meristem, including those in floral development. Because there is no evident alteration in the apical meristem, the *crl* mutation may affect aspects of development after initiation. Moreover, the mutation illustrates that not all alterations in the shoot apex can be attributed to alterations in the apical meristem.

Mutations Disrupting the Vegetative Shoot Apical Meristem

In contrast to the *crl* mutation, several other mutations identified as altering the vegetative shoot apex were found to also disrupt the apical meristem. Figure 7 shows the disruption in the shoot apex in the Forever young (Fey) mutant. Despite the perturbation of the shoot apex, cotyledons in Fey plants are normal (Figure 7C). In addition, the Fey hypocotyl and roots are also indistinguishable from the wild type (data not shown). This indicates that the primary lesion is specific to the shoot meristem and/or shoot apex. The alteration to the shoot apex is at times variable, but the first pair of leaf primordia is always initiated normally. The development of the first pair of leaf primordia is often not complete (Figures 7B and 7C). The Fey phenotype shown in Figures 7B and 7C is seen when the plants are grown at 25°C. However, when the plants are grown at 18°C the phenotype is less severe, but still distinguishable from the wild type. This suggests that either the *fey* mutation or the developmental process is temperature sensitive. When grown at 25°C, Fey plants make a variable number (two to seven) of abnormal leaf primordia and die without proceeding to inflorescence or floral development. When grown at 18°C, some Fey plants make flowers with the correct number and type of organs. However, development of each type of floral organ arrests prior to complete differentiation (data not shown).

Figure 8 shows that the *fey* mutation disrupts the shoot apex and shoot apical meristem. The mutant apical meristem has a more flattened shape than the wild type, and the cells are disorganized (Figure 8B). The disorganization in the meristem

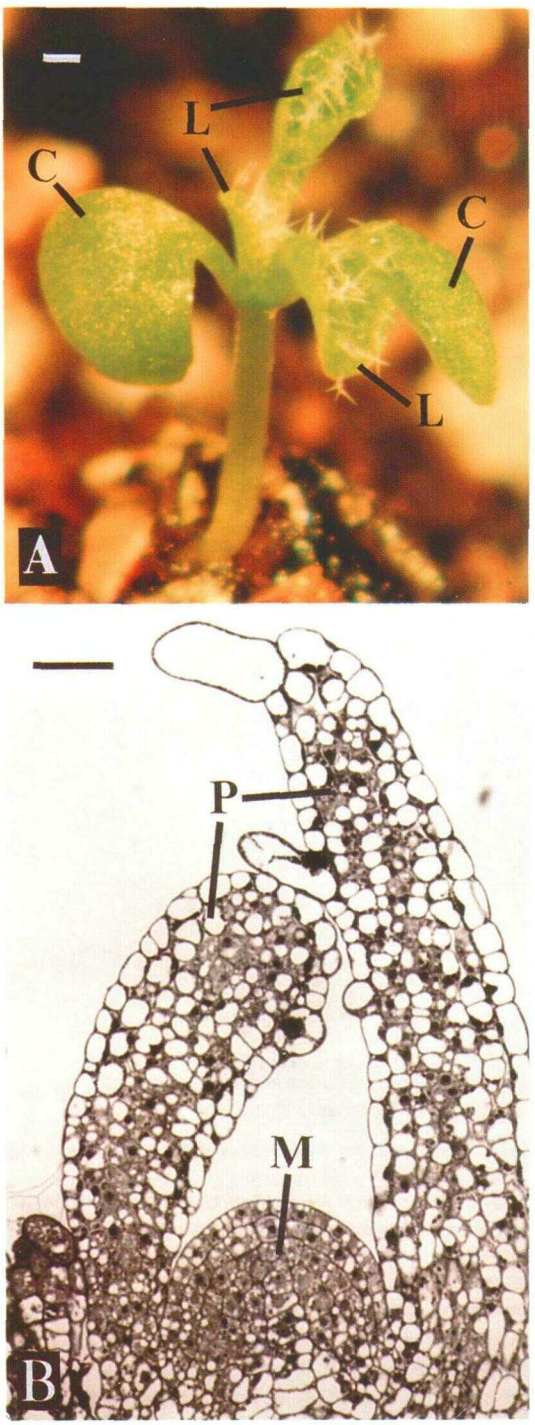

Figure 6. Abnormal Development in the Crl Shoot Apex.

(A) Photograph showing that the *crl* mutation affects both the leaves and cotyledons. Bar = 250 μm.

(B) Median section through 8-day-old Crl shoot apex. Cells in developing leaves show abnormal expansion. Bar = 25 μm.

C, cotyledon; L, leaf; M, meristem; P, primordia.

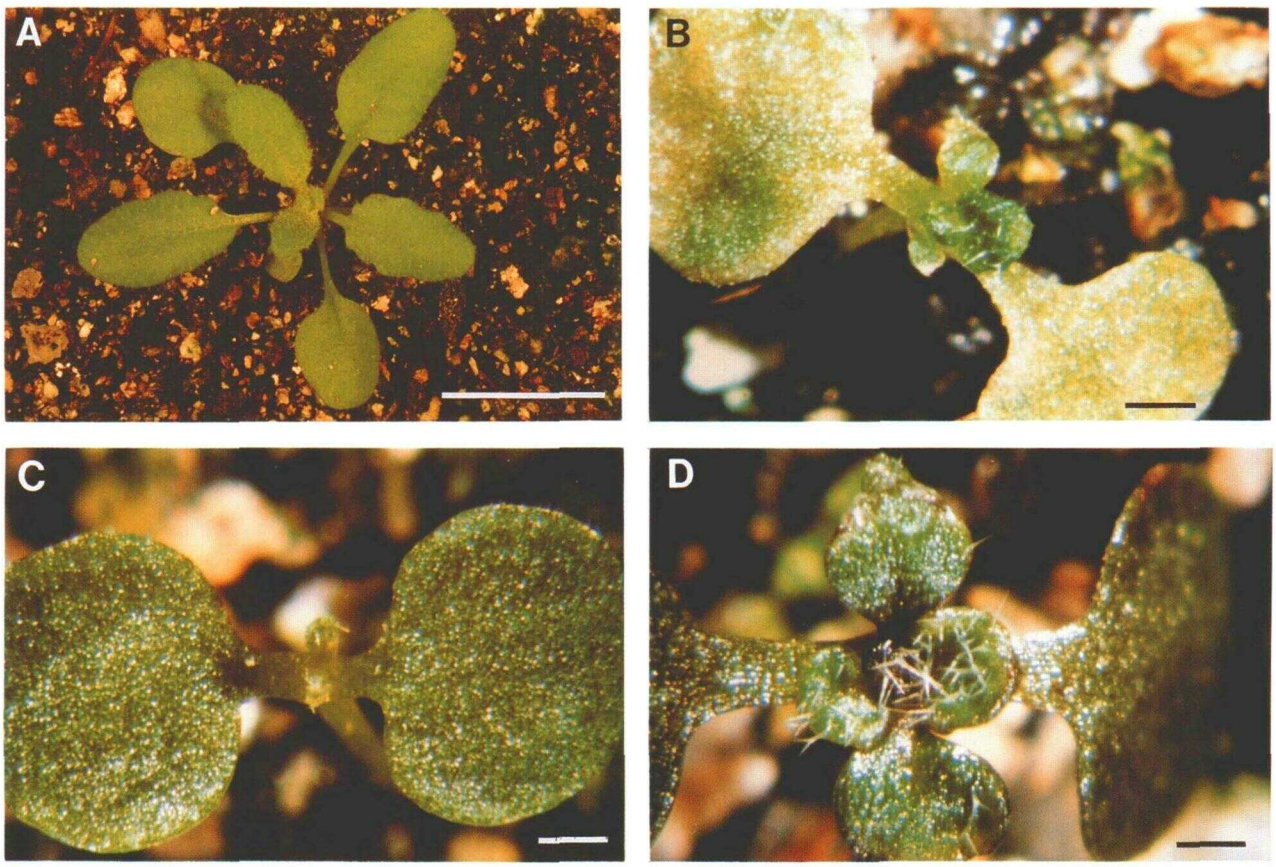

Figure 7. Abnormal Development in the Fey Shoot Apex.

(A) Wild-type plant, 17-day-old.
(B) Fey mutant, 17-day-old.
(C) 16-day-old Fey mutant grown at 25°C.
(D) 16-day-old Fey mutant grown at 18°C.
(A) Bar = 1 cm. **(B)** to **(D)** Bars = 0.5 mm.

cells produces a disruption in apical zonation. In addition, leaf primordia in the Fey mutant are initiated with too many cells. Older Fey primordia have abnormal shapes and expansion. The Fey mutant is not distinguishable from the wild type until after the first pair of leaf primordia are initiated. If the meristem does not function in embryogenesis, since zonation is not distinct until after the first pair of primordia are formed (Figures 1 and 3), the *fey* mutation may have direct effects on sequestering of cells to the peripheral zone to form leaf primordia.

Figure 9 shows the phenotypes of three additional mutants that disrupt the shoot apex. A macroscopic view of the Fully fasciated (Fuf) mutant shows no obvious disruption to the shoot apex other than an increase in organ (leaf) number (Figure 9B). (This mutation was generated with ethyl methanesulfonate [EMS] and was the kind gift of C. Somerville, Michigan State University, East Lansing.) The most distinct phenotype of the *fuf* mutation is a fasciated (fused and flattened) stem (Figure 9C). Like other mutations causing fasciation (e.g., *f158, clv1, clv2*; Kranz and Kirchheim, 1987), *fuf* has the most dramatic effect in the stem (Figure 9C), which is a product of the inflorescence meristem. However, Figure 10A shows that there is a dramatic increase in the size of the Fuf vegetative apical meristem, which has a diameter approximately twice that of the wild type (Figure 5B). Also, the Fuf apical meristem often had suggestions of cell divisions in L1 in planes other than anticlinal (Figure 10A). The Fuf mutant did not cause any detectable alteration in meristem zonation nor in the shape and differentiation of vegetative organs, but did produce club-shaped siliques.

Figures 9D and 10B show that the *disrupted* (*dip*) mutation also perturbs the shoot apex and apical meristem. When examined in section, the Dip apical meristem is noticeably misshapen and zonation in the apical meristem is not distinct (Figure 10B). Disrupted leaf shape is variable. At times the leaves are crinkled (Figure 9D), and at other times they are narrow and elongated with disorganized cells. Some leaf

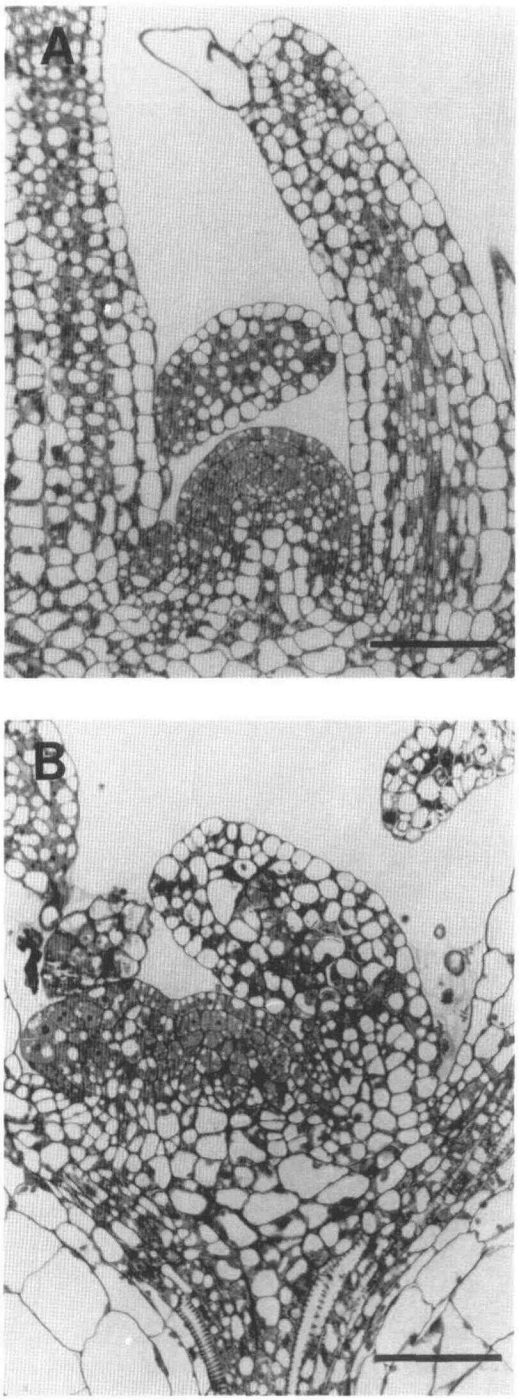

Figure 8. Median Sections through 8-Day-Old Wild-Type and Fey Shoot Apices.

(A) Wild-type shoot apex. Primordia 3 and 4 are located on either side of the apical meristem and at the base of the meristem; cells in the rib zone have begun to elongate.

(B) Fey shoot apex. The third leaf primordium (left) contains an abnormally high number of cells and zonation in the meristem is disrupted. Bars = 50 μm.

primordia in the Dip mutant develop without dorsiventral symmetry, whereas at other times there is no disruption in dorsiventrality but the leaf blade has a square shape with altered thickness. In addition to changes in the meristem and developing primordia, the *dip* mutation frequently, although not always, alters cotyledon shape.

Figures 9E, 10C, and 10D show the phenotype of the Schizoid (Shz) mutant, so named because multiple vegetative shoot apices are found. Sections through the shoot apex at 10 and 16 days (Figures 10C and 10D) show an alteration in the rib zone. At 10 days, both intracellular and intercellular alterations are seen in the rib zone. Rib zone cells in Shz are not in orderly files, and cytoplasm within these cells has an abnormal arrangement (Figure 10C). By 16 days, main stem cells, the interior of which are derived from the rib zone, show a severe necrosis (Figure 10D). In addition to the necrosis in the main stem, some lateral derivatives of the apical meristem also become necrotic (Figure 10D).

DISCUSSION

During early development, the vegetative shoot apical meristem produces the tissues and organs that will form the aboveground portion of the plant. In Arabidopsis, changes in the vegetative apical meristem predict changes in the developing shoot apex, which are summarized in Figure 11. The shoot apical meristem forms during embryogenesis. After 2 days of postembryonic development, the first pair of leaf primordia is initiated on opposite sides of the shoot apical meristem. At this stage, the meristem has a rectangular shape with bilateral symmetry. After 4 days of development, the first two leaf primordia become dorsiventral and a pair of stipules forms at their bases. The meristem is still bilaterally symmetrical and has a greater cell number at one end, giving it a trapezoidal shape. Leaf primordium three is initiated just prior to leaf primordium four at the wide end of the meristem and in a position opposite from where primordium four will form. The angle between the third and fourth leaf primordia approaches 180°. However, a plane between the third and fourth primordia is frequently not at 90° with respect to the plane of the first two leaf primordia (Figure 4). This slight skewing of the angles may be the onset of the transition from opposite to spiral phyllotaxy. After the formation of the fourth primordium, the meristem shape changes to a radially symmetrical dome, and all subsequent primordia are produced in a spiral phyllotaxy. The vegetative apical meristem proceeds through a sequential progression: bilateral rectangle to bilateral trapezoid to radially symmetrical dome. The dome-shaped meristem produces lateral organs in a repetitive pattern, unlike the short sequence seen from the bilateral meristem. The change in phyllotaxy is accompanied by a change in leaf shape or heterophylly. Early leaves are round and entire, whereas later leaves are more spatulate and serrated (Röbbelen, 1957). The overall processes defined here are often accompanied by subtle changes in cellular and

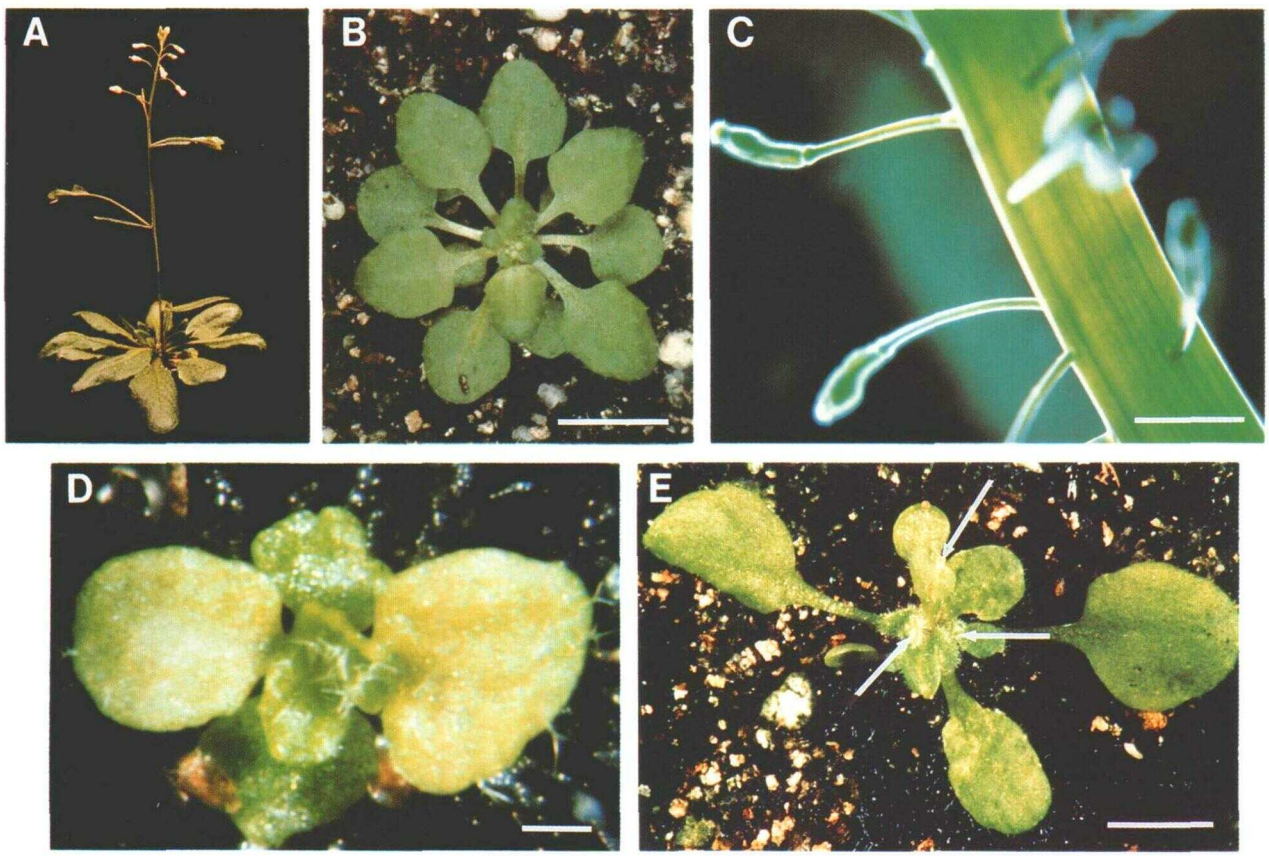

Figure 9. Additional Mutants Altering the Arabidopsis Shoot Apex.

(A) A 30-day-old wild-type (Columbia ecotype) plant.
(B) A 30-day-old Fuf mutant (Columbia ecotype) with increased numbers of leaves. Bar = 1 cm.
(C) Fasciated stem from a bolted Fuf mutant. Bar = 1 mM.
(D) Shoot apex of a 10-day-old Dip mutant. Bar = 0.5 mm.
(E) Shoot apex of a 26-day-old Shz mutant. Arrows indicate multiple shoot apices. Bar = 1 cm.

subcellular organization in the meristem. At the subcellular level, for example, the nucleolus appears centrally located within the nucleus of central zone cells, whereas in the peripheral zone cells, the nucleolus is often at one end of the nucleus.

After the dome-shaped shoot apical meristem is formed, a reiterative type of development starts for leaf initiation. In Arabidopsis plants grown under long-day conditions this may be only one repetition, whereas plants grown under short days may repeat the cycle numerous times prior to producing an inflorescence meristem. This repetitive process in vegetative development is not found in normal development from the floral meristem. It is interesting to note that the Agamous mutant (Yanofsky et al., 1990) not only changes organ identity, but also changes the pattern of organ initiation from sequential (sepal, petal, stamen, carpel) to repetitive (sepal, petal). In addition to the repetitive developmental pattern, the pattern of initiation from a vegetative meristem is also different in that a unit (a phytomer consisting of a leaf, an internode, and an axillary bud), and not a single organ, is formed.

It is interesting to note that the stipules for leaves one and two are apparently crushed with the development of the third and fourth primordia (Figure 4B). A model to explain trichome differentiation proposes that a morphogenetic gradient is established by expression in stipules (Oppenheimer et al., 1991). The apparent crushing of stipules during shoot development may add insight to this model.

The mutations affecting vegetative development described in Figures 6 through 10 offer a unique opportunity to describe early processes in the shoot apex in genetic terms. The mutations we described fall into at least three classes: (1) lesions in the shoot apex that do not show an apparent alteration in the shoot apical meristem, but affect leaf primordia (*crl*); (2) lesions in the apical meristem that also (directly or indirectly) alter primordia (*dip*, *fey*, and *shz*); and (3) lesions in the apical meristem that alter meristem size and leaf number but not leaf

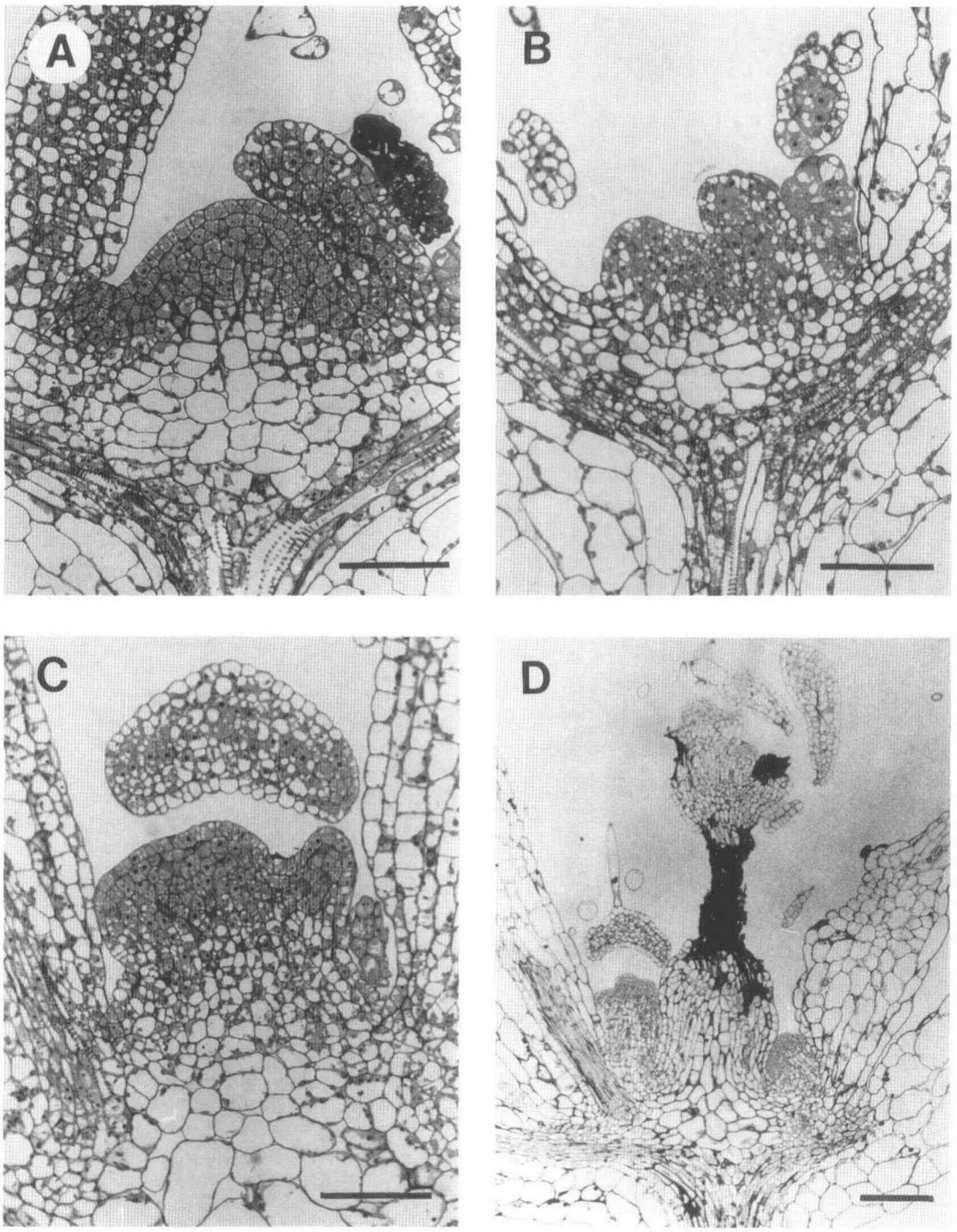

Figure 10. Sections through Fuf and Shz Mutants.

(A) Near median section through 7-day-old Fuf meristem. The meristem is enlarged but has no apparent disruption in zonation. An example of a nonanticlinal division in L1 is at the distal end of the apical meristem. (Comparable section through 7-day-old wild-type meristem is in Figure 5B.)
(B) Near median section through 7-day-old Dip meristem.
(C) Near median section through 10-day-old Shz meristem. Cells at the base of the meristem, the rib zone, have both intracellular and intercellular abnormalities.
(D) Section through 16-day-old Shz shoot apex. The main stem is necrotic, and the axillary meristems have started to grow.
(A) to **(C)** Bars = 50 μm. **(D)** Bar = 100 μm.

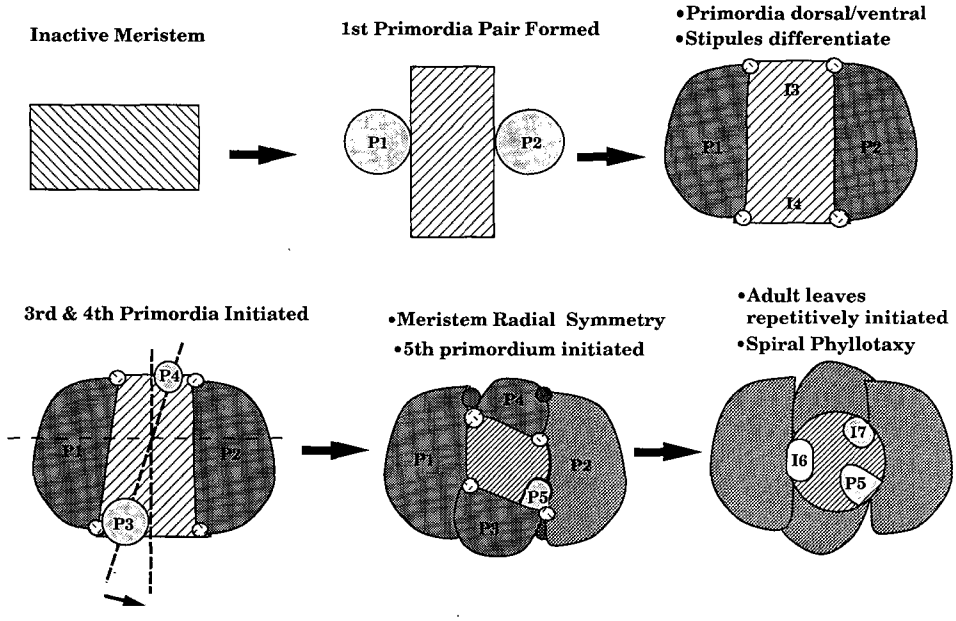

Figure 11. Diagrammatic Summary of Changes in Arabidopsis Vegetative Apical Meristem.

The juvenile meristem is a bilaterally symmetrical rectangle. The first pair of leaf primordia (P1 and P2) are initiated on opposite sides of the meristem. The young primordia initially have a radial symmetry. Two days later, the primordia develop dorsiventral symmetry and a pair of stipules form at the base of each primordium. Cells for the second pair of leaf primordia (I3 and I4) are formed in the peripheral zone. The meristem enlarges at one end and assumes a trapezoidal shape yet still has bilateral symmetry. The third primordium (P3) is formed at the wide end of the trapezoid, and the fourth primordium (P4) is formed approximately opposite the third. At 7 days, the meristem undergoes a dramatic change and becomes a radially symmetrical dome. The fifth primordium (P5) forms from the meristem, and all subsequent primordia are initiated in spiral phyllotaxy. Since early Arabidopsis development follows a sequence, the leaf primordia are described in increasing order (e.g., P3 is the third primordium formed, P4 is the fourth leaf primordium formed).
I, incipient leaf primordia.

morphology (*fuf*). The fact that the *crl* mutation affected the shoot apex without an apparent effect on the meristem supports the distinction between the shoot apex and apical meristem (Cutter, 1965). Although the *crl* mutation did not cause disruption in the meristem comparable to *fey* or *dip*, it is still possible that the lesion acts in the apical meristem but is not detectable by light microscopy. Nonetheless, there is a clear distinction between mutants such as Crl, which do not involve visible meristem alterations directly, and mutants such as Fey, which do involve visible alterations in the apical meristem.

Even at this preliminary stage, some insight into vegetative development can be obtained from these mutants. For example, the Shz mutant (Figure 10C) primarily disrupted the rib zone of the apical meristem. However, this lesion was not seen in the axillary meristems. This could be a result of either tight temporal regulation of the *shz* gene product or a difference between the main apical meristem and axillary meristems. Because *shz* alters derivatives of the main floral meristem as well as the vegetative meristem (Figure 10D), a tight temporal regulation seems unlikely. If there is a regulatory difference between main and axillary meristems, the *shz* mutation should provide a valuable tool to study an issue unapproachable through other means. Presumably, the necrosis seen in a Shz stem at 16 days prevents transmission of an inhibitory signal from the main apex (or the signal is not properly made) that leads to release of axillary buds and the Shz phenotype.

The fasciation in Fuf represents a true fasciation (alteration in one growing point) rather then a connation (alteration in two or more growing points) (Gorter, 1965). Enlargement of the vegetative meristem in Fuf does not appear to alter zonation (Figure 10A). Because previous studies on meristems suggest that there is communication between meristem zones and between the meristem and a primordium (Steeves and Sussex, 1989), the enlarged Fuf meristem may allow insight into these processes. Fasciated plants have been known for centuries, and it is interesting to note that an inhibitor of auxin transport was correlated with stem fasciation (Gorter, 1965). The alteration seen in Fuf L1 cells to a plane other than strictly anticlinal (Figure 10A) was hypothesized by Gorter (1965) to be the origin of fasciation. This alteration could explain both the enlarged apical meristem in Fuf and the increase in organ number.

Mutants such as Fuf may offer insight into how the position of primordia on the apical meristem is determined. Over the years, many theories on the precise positioning of leaf primor-

dia have been proposed. One theory suggests that primordia arise where there is the first available space (Snow and Snow, 1933). The Fuf apical meristem is greatly enlarged, yet shows no gross phyllotactic abnormality, suggesting that factors other than available space contribute to leaf positioning. More definitive data are currently being collected on leaf positioning using both the Fuf and Fey mutants. This should allow us to test the various models for leaf positioning.

In the Fey (and Dip) mutants that disrupt leaf primordia, there is always an accompanying disruption in the apical meristem. Three explanations are possible for this scenario. First, it is possible that the mutation disrupts a function common to both the apical meristem and leaf primordia. Second, because the meristem produces the leaf primordia, it is possible that cells that are disrupted in the meristem produce disrupted leaf primordia. Third, it is possible that there is some type of communication between the meristem and primordia. The latter possibility was suggested by I. M. Sussex from surgical experiments (Sussex, 1951). Further analysis of Fey and Dip, and mutants such as Fuf, in which the distances between the meristem and primordia are altered, will allow examination of the hypothesis that communication between the apical meristem and primordia is necessary for proper development of leaves.

The fact that most Fey mutants grown at 25°C die without proceeding to inflorescence or floral development is particularly intriguing. In some near median sections of Fey we have seen a dramatic decrease in the meristem size, and in some Fey SEMs we could not detect a shoot apical meristem. Because Fey leaf primordia contain an abnormally large number of cells (Figure 8B), one explanation is that the apical meristem is simply "used up" in primordia formation. The temperature sensitivity seen in Fey has been reported for other Arabidopsis mutants. A recent description of temperature sensitivity in floral mutants has suggested that this could be either from the developmental process or the mutation (Coen and Meyerowitz, 1991). At this time, either explanation is possible for Fey.

Previous studies have presented analyses of developmental changes in floral meristems and, more recently, inflorescence meristems in Arabidopsis (Smyth et al., 1990; Schultz and Haughn, 1991; Shannon and Meeks-Wagner, 1991). Mutations in vegetative development offer the unique opportunity to analyze how processes early in development do or do not influence those of later development. More detailed analyses of these mutations, their interactions with floral mutations, and isolation of the disrupted genes are in progress.

METHODS

Mutant Isolation

The mutations were isolated by screening soil-grown plants between 10 and 16 days old as a part of a large-scale screen at the Du Pont Company, Wilmington, DE (Feldmann, 1991). The Fully fasciated (Fuf) mutant was isolated from an ethyl methanesulfonate (EMS) population (Columbia ecotype) and is the kind gift of C. Somerville.

Plant Materials

Unless noted, all plant material is of the ecotype Wassilewskija. Mature seed of *Arabidopsis thaliana* were cold treated to synchronize early development. Without such treatment, development varied by as much as 2 days. For material used in sectioning, mature seeds were imbibed for 12 hr at 4°C on the surface of soil and then transferred to a growth chamber with the temperature maintained at 22°C. For material used for SEM, seeds were imbibed at 4°C for 48 hr. The age of the plants was recorded from the time of their transfer to a growth chamber. A 16-hr/8-hr light/dark cycle maintained with a combination of cool white and grow-lux bulbs providing 60 to 90 μmol m^{-2} sec^{-1} was employed.

SEM and Histology

Seedlings were fixed in 2% glutaraldehyde in 0.07 M sodium phosphate/potassium phosphate buffer, pH 6.84, at 4°C for 24 hr. For SEM, postfixation was in 1% osmium tetroxide for 24 hr at 4°C. Seedlings were rinsed in buffer, dehydrated in a graded ethanol series, and critical point dried in CO_2 in a pressure bomb (P, 1200 psi; temperature, 37°C; model No. E3000, Polaron, Waterford, England). Seedlings were mounted on aluminum stubs and sputter-coated with 28 nm of gold. Developing organs were removed using glass needles, and the samples were again coated with gold. Samples were examined at an accelerating voltage of 10 kV in an ISI-60 scanning electron microscope. Photographs were taken on Polaroid-type 55 (ISO 50) high-resolution film. Histology was performed by dehydrating samples in an ethanol series and embedding them in Spurr resin (Electron Microscopy Sciences, Fort Washington, PA). One-micron sections were cut with glass knives and stained in a solution containing 1% toluidine blue and 1% sodium borate. Sections were photographed using a camera (model No. FX-35WA, Nikon) and an Axioscope (Zeiss).

Primordium cell number was determined by first calculating a primordium's volume with a parabolic approximation, then dividing this by the volume of an average cell. Meristem cell number was estimated by counting cells in each of the three planes.

ACKNOWLEDGMENTS

We thank Rosemary Walsh for help with the SEM, Richard Cyr for advice and use of a Zeiss microscope, Dan Cosgrove for help with glass dissection needles, Bruce Link for help with calculations, and Bridget Stemberger for advice with embedding and sectioning.

Received January 7, 1992; accepted April 15, 1992.

REFERENCES

Besnard-Wibaut, C. (1977). Histoautoradiographic analysis of thermoinductive processes in the shoot apex of *Arabidopsis thaliana*

(L.) Heynh. vernalized at different stages of development. Plant Cell Physiol. **18,** 949–962.

Bowman, J.L., Drews, G.N., and Meyerowitz, E.M. (1991). Expression of the Arabidopsis floral homeotic gene *AGAMOUS* is restricted to specific cell types late in flower development. Plant Cell **3,** 749–758.

Christianson, M.L. (1986). Fate map of the organizing shoot apex in *Gossypium.* Am. J. Bot. **73,** 947–958.

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

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

Cutter, E.G. (1965). Recent experimental studies of the shoot apex and shoot morphogenesis. Bot. Rev. **31,** 7–113.

Esau, K. (1977). Anatomy of Seed Plants, 2nd ed. (New York: John Wiley and Sons).

Feldmann, K.A. (1991). T-DNA insertion mutagenesis in *Arabidopsis:* Mutational spectrum. Plant J. **1,** 71–82.

Gorter, C.J. (1965). Origin of fasciation. In Encyclopedia of Plant Physiology, W. Ruhland, ed (New York: Springer-Verlag), pp. 330–351.

Kranz, A.R., and Kirchheim, B. (1987). Genetic Resources in *Arabidopsis.* Arab. Infor. Service **24,** 1–47.

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

Miksche, J.P., and Brown, J.A. (1965). Development of vegetative and floral meristems of *Arabidopsis thaliana.* Am. J. Bot. **52,** 533–537.

Oppenheimer, D.G., Herman, P.L., Sivakumaran, S., Esch, J., and Marks, M.D. (1991). A *myb* gene required for leaf trichome differentiation in *Arabidopsis* is expressed in stipules. Cell **67,** 483–493.

Poethig, R.S. (1990). Phase change and the regulation of shoot morphogenesis in plants. Science **250,** 923–930.

Röbbelen, G. (1957). Über heterophyllie bei *Arabidopsis thaliana* (L.) Heynh. Ber. dt. bot. Ges. **70,** 39–44.

Schultz, E.A., and Haughn, G.W. (1991). *LEAFY,* a homeotic gene that regulates inflorescence development in Arabidopsis. Plant Cell **3,** 771–781.

Shannon, S., and Meeks-Wagner, D.R. (1991). A mutation in the Arabidopsis *TFL1* gene affects inflorescence meristem development. Plant Cell **3,** 877–892.

Smyth, D.R., Bowman, J.L., and Meyerowitz, E.M. (1990). Early flower development in *Arabidopsis.* Plant Cell **2,** 755–767.

Snow, M., and Snow, R. (1983). Experiments on phyllotaxis. II. The effect of displacing a primordium. Phil. Trans. Roy. Soc. Lond. B **222,** 353–400.

Steeves, T.A., and Sussex, I.M. (1989). Patterns in Plant Development, 2nd ed. (New York: Cambridge University Press).

Sussex, I.M. (1951). Experiments on the cause of dorsiventrality in leaves. Nature **167,** 651–652.

Ursin, V.M., Irvine, J.M., Hiatt, W.R., and Shewmaker, C.K. (1991). Developmental analysis of elongation factor-1a expression in transgenic tobacco. Plant Cell **3,** 583–591.

Vaughan, J.G. (1955). The morphology and growth of the vegetative and reproductive apices of *Arabidopsis thaliana* (L.) Heynh., *Capsella bursa-pastoris* (L.) Medic. and *Anagallis arvensis* L. J. Linn. Soc. Bot. **55,** 279–300.

Wardlaw, C.W. (1957). On the organization and reactivity of the shoot apex in vascular plants. Am. J. Bot. **44,** 176–185.

Yamamoto, Y.T., Taylor, C.G., Acedo, G.N., Cheng, C.-L., and Conkling, M.A. (1991). Characterization of *cis*-acting sequences regulating root-specific gene expression in tobacco. Plant Cell **3,** 371–382.

Yanofsky, M.F., Ma, H., Bowman, J.L., Drews, G.N., Feldmann, K.A., and Meyerowitz, E.M. (1990). The protein encoded by the *Arabidopsis* homeotic gene *agamous* resembles transcriptional factors. Nature **346,** 35–39.