

Formation of the shoot apical meristem in *Arabidopsis thaliana*: an analysis of development in the wild type and in the *shoot meristemless* mutant

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SUMMARY

The primary shoot apical meristem of *Arabidopsis* is initiated late in embryogenesis, after the initiation of the cotyledons. We have identified a gene, called *SHOOT MERISTEMLESS*, which is critical for this process. *shoot meristemless* mutant seedlings lack a shoot apical meristem but are otherwise healthy and viable. The anatomy of mutant embryos demonstrates that the *shoot meristemless-1* mutation completely blocks the initiation of the shoot

apical meristem, but has no other obvious effects on embryo development. The failure of *shoot meristemless* tissue to regenerate shoots in tissue culture suggests that this gene regulates adventitious shoot meristem formation, as well as embryonic shoot meristem formation.

Key words: meristem, *SHOOT MERISTEMLESS*, plant development, *Arabidopsis*, embryogenesis

INTRODUCTION

Almost all of the tissue in a mature plant is ultimately derived from specialized groups of cells, called meristems, that reside at the tips of roots and shoots. Shoot apical meristems (SAMs) are responsible for elaborating the above ground portions of the plant, which include stems, leaves and flowers, whereas root apical meristems generate the extensive underground network of roots necessary for water and mineral uptake.

The shoot apical meristem consists of a small mound of densely cytoplasmic, undifferentiated, dividing cells. Based on several criteria, the cells in the meristem can be classified as stem cells (Potten and Loeffler, 1990). Among these are the ability to proliferate, to replace themselves, to give rise to a variety of differentiated cell types, and to regenerate a new meristem if damaged (Sussex, 1952).

The shoot apical meristem of angiosperm plants is organized into two regions - the tunica and the corpus (Schmidt, 1924). The tunica consists of one or more cell layers that cloak the underlying corpus. The *Arabidopsis* vegetative meristem consists of two tunica layers overlying a shallow corpus (Vaughn, 1955; Medford et al., 1992). The organization of the meristem into these two distinct regions is a consequence of differences in the orientation of cell division. Cell divisions in the tunica are principally anticlinal¹, thus maintaining the layered arrangement of the tunica.

The cells in the corpus on the other hand may divide anticlinally, periclinally or obliquely resulting in a jumbled arrangement of cells. Although the tunica and corpus have been recognized as anatomically distinct regions since the early part of this century (Schmidt, 1924) the significance of this organization to meristem function, if any, is unknown.

Shoot apical meristems of angiosperm plants form under three distinct sets of conditions. The first of these is during embryogenesis when the primary shoot apical meristem is formed. The second condition is in the axils of leaves where lateral shoot apical meristems are formed. The third is adventitiously, i.e. from a portion of the plant that when unperturbed does not give rise to a SAM. Examples of adventitious SAM formation are the formation of SAMs from the petioles of isolated leaves in African violets and from *Arabidopsis* roots cultured in vitro (Valvekens et al., 1988).

Previous studies of the mechanism of meristem initiation have focused on the growth factors that regulate adventitious meristem formation in vitro. Numerous studies on many different species have shown that a high auxin:cytokinin ratio promotes root development and a low auxin:cytokinin ratio promotes shoot development (e.g. Skoog and Miller, 1957). However, the basis of this effect and the mechanism by which the initiation and identity of meristems is regulated in vivo are still unknown.

Although embryogenesis has been described in a wide variety of plants, little attention has been paid to the initiation of the shoot apical meristem. In order to understand the mechanism of meristem initiation we have char-

¹The terms anticlinal and periclinal are used to indicate the orientation of cell division. Anticlinal divisions are those in which the spindle is oriented parallel to the nearest surface. Periclinal divisions are those in which the spindle is perpendicular to the nearest surface.

acterized the developmental anatomy of meristem initiation in *Arabidopsis thaliana* and screened for mutations that affect this process. Here we describe the initiation of the shoot meristem in wild-type *Arabidopsis thaliana* embryos and describe the effects of a recessive, EMS induced mutation in the *SHOOT MERISTEMLESS (STM)* gene on this process. We show that this mutation specifically blocks the initiation of the SAM without affecting the growth or viability of other portions of the embryo.

MATERIALS AND METHODS

Plant growth conditions

Unless otherwise noted, plants were grown in soil (Metromix 200) under 24 hour cool white fluorescent light at $26 \pm 3^\circ\text{C}$.

Histology

Siliques (for embryos) or seedlings were fixed in 3% glutaraldehyde, 1.5% acrolein, 1.6% paraformaldehyde and postfixed in 1% osmium tetroxide. Fixed material was dehydrated through an ethanol series and embedded in Spurr's medium. 1 μm sections were cut and stained with 0.5% methylene blue in 0.5% borate.

Stages of embryogenesis were identified using the following criteria. The onset of heart stage was defined as the point when the cotyledons first extended above the apex. Torpedo stage was defined as beginning when the cotyledons accounted for at least one third of the length of the embryo. Embryos were assigned to the bending cotyledon stage if the cotyledons had begun to bend over the apex. Fully formed embryos were those in which the cotyledons had completely folded over the apex and extended all the way to the root tip.

SAM development was reconstructed from longitudinal sections of a total of 10 globular stage, 15 heart stage, 8 torpedo stage, 18 bending cotyledon stage and 27 fully formed embryos. 5/15 heart stage embryos had two layers of cells at the presumptive apex, 3/15 had three layers and 7/15 were in transition between two and three. 6/8 torpedo stage embryos had formed three layers of cells at the presumptive apex, 1/8 was in transition between 2 and 3 and in 1/8 the layering was not clearly discernible. All of the 45 more advanced embryos had three well-formed layers that could be divided into a tunica and corpus region (or layers that were forming a tunica and a corpus) at the shoot apex. A periclinal division was seen in the upper hypodermal layer of 4 of these. Periclinal divisions were never observed in the epidermis.

Genetics

Seeds of ecotype Landsberg *erecta* were mutagenized by soaking them for 8 hours in 0.4% EMS in 100 mM phosphate buffer (pH 7). The self-progeny of individual plants grown from mutagenized seed ($n=1668$) were harvested, planted in soil and screened 4-7 days after germination. Wild-type siblings from pots containing candidate mutants were used as a source of heterozygotes to propagate the seedling lethal mutations thus generated.

The *stm-1* mutation was mapped to linkage group I by analyzing recombinants in the self progeny of *stm-1 chl/++* parents. Seedlings homozygous for the *chl* mutation are yellow-green. From a total of 102 self-progeny, 12 yellow non-*stm* and 6 green *stm* seedlings were counted. This corresponds to a recombination frequency (p) of 19.5% (calculated as $p=1-1-2R$, where R is the fraction of progeny with recombinant phenotypes (Brenner, 1974)).

Tissue culture

Shoots were regenerated from root explants according to Valvekens et al. (1989). Roots from *stm-1* seedlings or their wild-type siblings were cut into small pieces and put onto callus

inducing medium (0.5 mg/l 2,4-D; 0.05 mg/l kinetin). They were transferred after 9 days to shoot-inducing medium (7 mg/l N^6 -(2-isopentenyl)adenine; 0.05 mg/l indole-3-acetic acid). Cultures were grown at 24°C under 16 hours light/8 hours dark cycles. The cultures were scored after 5 weeks for the presence of shoots.

RESULTS

Initiation of the shoot apical meristem during wild-type embryogenesis in *Arabidopsis*

Although general embryonic development has been described in *Arabidopsis thaliana* (Mansfield and Briarty, 1991), the genesis of the shoot apical meristem was not a focus of these studies. Thus, to obtain a reference point against which to compare mutant development, it was necessary to describe the origin of the shoot apical meristem during embryogenesis. Since it has not yet been possible to follow individual cells in living *Arabidopsis* embryos, the analysis presented here relies on the observation of fixed and sectioned embryos at progressive stages of development. Our analysis begins at the globular stage of embryogenesis and is confined to the median longitudinal plane of the embryo.

Several factors make ascribing cell fates based on histology more reliable in plants than in animals. (1) The lack of cell migration in plants. The plant cell wall poses a barrier to migration. Thus, the pattern of cell walls creates a 'frozen' record of cell divisions. Although this record may become obscured through later cell expansion, certain walls retain a distinctive morphology and can be used as landmarks in the analysis of development. (2) The placement of new cell walls relative to older walls. New cell walls typically meet older walls along their faces rather than their edges (see Lloyd, 1991, for a discussion of the rules that govern the placement of cell walls). As a consequence the order in which walls have been laid down can often be inferred. (3) The regularity (and in some cases the invariance) of cell division patterns during embryogenesis in the Brassicaceae. Members of this family (including *Arabidopsis*) have long been known to exhibit very predictable patterns of cell divisions during embryogenesis (Souèges, 1914).

The *Arabidopsis* zygote divides transversely to yield two cells - a small densely cytoplasmic terminal cell and a larger, vacuolate basal cell (Mansfield and Briarty, 1991). These cells subsequently undergo a stereotyped pattern of cell division to produce a globular stage embryo consisting of a spherical embryo proper and a filamentous suspensor (Fig. 1A). The embryo proper is derived from the terminal cell and produces nearly all of the mature embryo. The suspensor arises from the basal cell via a series of transverse divisions. With the exception of the cell closest to the embryo proper, the hypophysis (Fig. 1A), the suspensor will not contribute to the embryo.

At the globular stage (Fig. 1A), the embryo proper can be divided into an upper and a lower hemisphere along the boundary produced by the first set of transverse divisions of the embryo proper. This boundary between the upper and the lower hemispheres provides a landmark set of walls that can be recognized in the central portion of the embryo throughout embryogenesis. In Fig. 1 this boundary is indicated throughout by a bold line. This boundary will ulti-

mately subtend the cells of the SAM and overlie the vascular cells of the hypocotyl (Fig. 1O).

The SAM is derived from a subset of the cells in the upper hemisphere of the globular stage embryo. At this stage, the upper hemisphere consists of two cell layers - an epidermal layer and a hypodermal layer. During the transition from the late globular to the torpedo stage, the upper hemisphere becomes stratified into three layers at the apex (Fig. 1C-J). This is a consequence of periclinal, or transverse, divisions in the hypodermal layer. Thus, by the late torpedo stage of embryo development, the central part of the upper hemisphere consists of three layers at the apex overlying a core of provascular cells. We refer to the three layers as the epidermal layer and the upper and lower hypodermal layers (in keeping with Sharman's (1945) description of lateral meristem development in couch grass).

The SAM arises from these three layers of cells in the torpedo stage embryo. As the cotyledons grow out and fold over the embryo, the three-layered region between them broadens and forms the SAM (Figs 5A,B, 1M). Cell division is responsible for this widening, since the apex is 3-4 cells in diameter at the torpedo stage and 7-8 cells in diameter in the fully formed embryo. Cells in the epidermal and upper hypodermal layer divide anticlinally, whereas cells in the lower hypodermal layer display a mixture of periclinal, anticlinal and oblique divisions (Fig. 1M,N). Storage granules accumulate throughout the embryo but are excluded from the SAM. The boundary of the SAM becomes increasingly well defined towards the end of embryogenesis as the size of the storage granules in the surrounding cells increases (Figs 1O, 4A). Figs 1O and 4A show that the embryonic SAM is slightly domed in the fully formed embryo. This has been confirmed by examination of whole-mount embryos viewed with DIC optics.

The stratified configuration of the embryonic meristem is perpetuated in the seedling meristem after germination as regions recognized in classical plant anatomy as the two tunica layers and the central corpus (Fig. 2A). Since no evidence of extensive periclinal divisions in either the epidermal or the upper hypodermal layer has been observed in young seedlings (2 days postgermination, Fig. 2A) and since plant cells do not move relative to one another, we conclude that the upper and lower tunica layers are direct derivatives of the epidermal and upper hypodermal layers of the embryo, respectively, and that the corpus is a derivative of the lower hypodermal layer.

In summary, the patterns of cell division that characterize the vegetative meristem (i.e. anticlinal divisions in the tunica layers and mixed cell division planes in the corpus) are apparent first after the torpedo stage of embryogenesis. These observations suggest that the SAM is initiated at or after the torpedo stage of embryogenesis. This is a relatively late point in embryogenesis. By the time the SAM appears as a histologically distinct entity, the root primordium is near fully formed, the cotyledons and provascular are readily distinguishable, and all of the tissues of the hypocotyl are in place.

The *SHOOT MERISTEMLESS* locus

Seedlings homozygous for the *shoot meristemless-1* mutation (*stm-1*) completely lack a SAM, but are otherwise

normal. Mutant plants can be identified four days postgermination when the first two leaves initiated by the SAM are visible in wild-type plants but are absent in *stm-1* seedlings (Fig. 3). Serial sections through *stm-1* seedlings 2 days postgermination ($n=6$) reveal no evidence of a SAM (Fig. 2C), nor is there any evidence of a SAM in mature *stm-1* embryos prior to germination (Fig. 4B). In all wild-type embryos examined ($n=50$) the SAM is well developed by this stage (Fig. 4A). Cells in the position of the SAM in *stm-1* seedlings are essentially indistinguishable from surrounding cells in the hypocotyl and cotyledons, although they may be somewhat smaller in size (Fig. 2).

The configuration of cells in the apical position of *stm-1* end stage embryos and young seedlings is similar to that seen in torpedo stage embryos (the stage just prior to that when a SAM first becomes histologically distinguishable; Figs 2, 4). Similar to wild-type torpedo stage embryos, the apical region of *stm-1* seedlings is three cell layers deep and 3-4 cells in breadth. The fact that the terminal phenotype of *stm* mutants resembles an earlier stage in normal development suggests strongly that the *stm-1* mutation causes a block in SAM development at or just after the torpedo stage of embryogenesis. To test this hypothesis, self-progeny embryos from parents of genotype *stm-1/+* were analyzed. (1/4 of these embryos are expected to be homozygous for *stm-1*). If the defect in *stm-1* embryos is a block at or just after the torpedo stage, then torpedo stage *stm* embryos should not be distinguishable from the wild type. In agreement with this prediction, 17/17 torpedo stage embryos examined appeared to be fully normal.

The first indication of a *stm* phenotype was seen in bending cotyledon stage embryos. 4/17 bending cotyledon stage embryos were distinguishable as *stm* mutants. Whereas in the wild-type embryo at this stage, a SAM is clearly starting to form, in the *stm* mutants, the arrangement of cells at the apex remains the same as in the torpedo stage embryo (Fig. 5). Specifically, three differences between wild-type and mutant embryos can be detected. These are: (1) the position of the fold formed by the uppermost cotyledon as it bends over the embryo; (2) the radius of curvature of the walls that separate the three layers of cells at the apex; (3) the number of cells showing an absence of intracellular bodies as determined by DIC optics.

In the wild-type bending cotyledon stage embryo, the fold that the uppermost cotyledon makes as it bends over the embryo is off center with respect to the long axis of the hypocotyl. (The hypocotyl vasculature provides a reference for center.) This reflects the fact that the region that will give rise to the SAM has begun to broaden. Indeed, in the end-stage embryo, this fold is seen to be adjacent to the SAM (Figs 1, 4). In the *stm* mutant embryo, this fold is at the center of the hypocotyl axis.

The radius of curvature of the walls that separate the three apical layers has increased at the partially bent stage relative to the torpedo stage. The boundaries between these layers flatten as the SAM develops (Figs 1, 5). This presumably reflects the expansion of cells in the lower hypodermal layer that pushes upwards on the overlying layers. The radius of curvature does not appear to change in the *stm* mutant embryo and the boundaries between these cell layers remain acutely curved as in the torpedo stage embryo (Fig. 5).

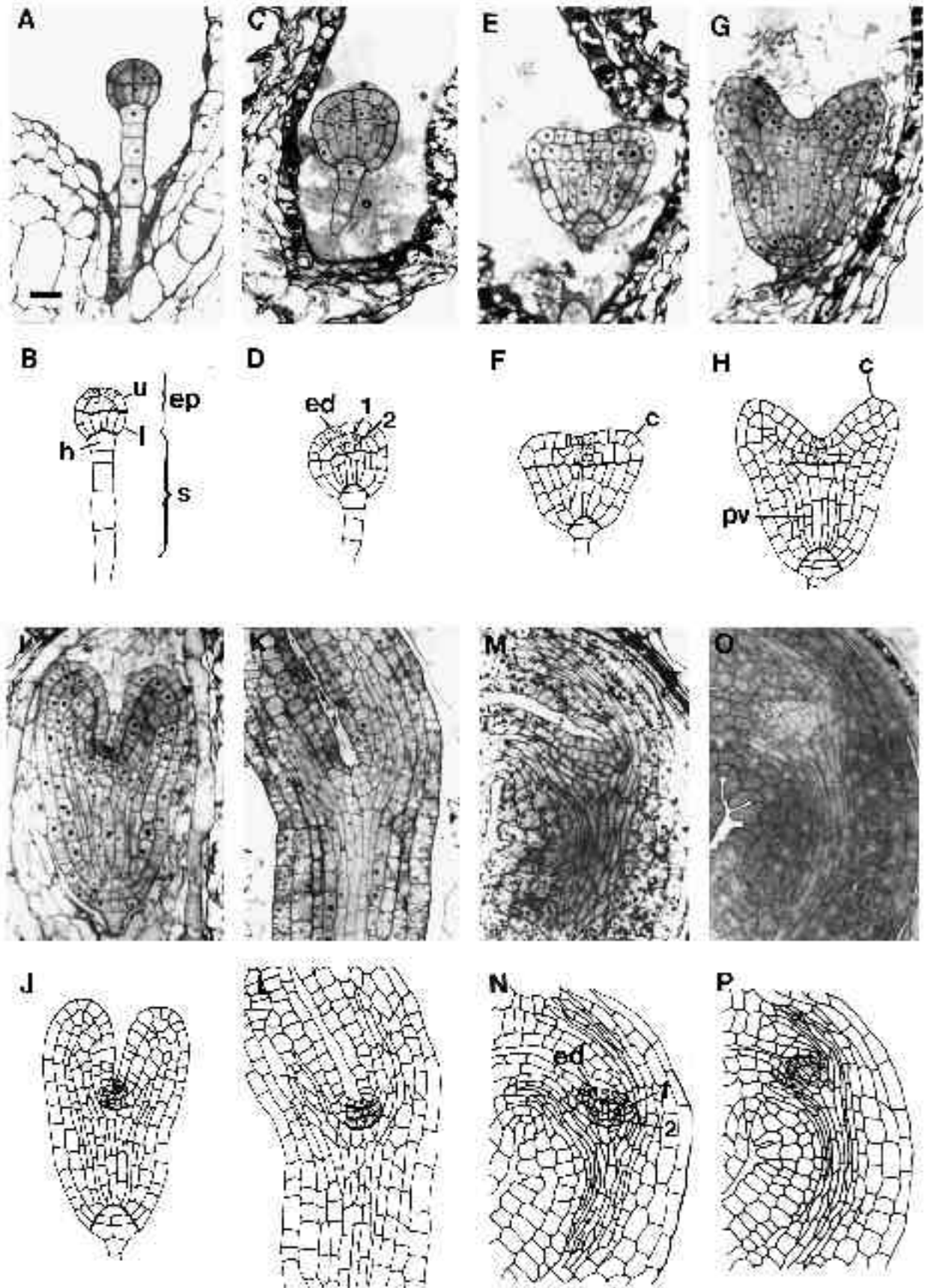


Fig. 1

Fig. 1. Development of the SAM in the wild-type *Arabidopsis* embryo. (A) Globular-stage embryo. (B) Tracing of A. The embryo proper (ep) can be divided into an upper (u) and a lower (l) hemisphere. The bold line indicates the boundary produced by the first set of transverse divisions of the embryo proper. Some, but not all, of the descendants of the stippled cells in the northern hemisphere will give rise to the shoot apical meristem. Only one of the cells derived from the suspensor (s), the hypophysis (h), will generate a portion of the embryo. (C) Late globular embryo. (D) Tracing of C. The bold line again indicates the boundary between the two hemispheres of the embryo. Stippling indicates cells some of whose descendants will take part in forming the SAM. Cells in the hypodermal layer have divided to generate three layers at the presumptive shoot apex, epidermal (ed), upper hypodermal (1) and lower hypodermal (2). (E) Early heart stage embryo. (F) Tracing of E. Cotyledons (c) extend just above presumptive SAM. Boundary between upper and lower hemispheres is still readily discernible across embryo and is marked with a bold line. In this individual (and in G), the hypodermal layer has not yet split completely into two layers. (G) Heart stage embryo. (H) Tracing of G. Boundary between upper and lower hemispheres is no longer easily discernible in lateral regions of embryo where it separates cells that will participate in forming the SAM (derived from the upper hemisphere) from those of the provascular tissue (derived from the lower hemisphere). At this stage it is uncertain how many cells in the lateral dimension will give rise to the SAM. We have only stippled those cells that are the most central, recognizing that cells just lateral to those stippled may also contribute descendants to the SAM. c, cotyledon; pv, provascular tissue. (I) Early torpedo stage embryo. (J) Tracing of I. (K) Early bending cotyledon stage embryo. The cotyledons have just begun to fold over the embryo. (L) Tracing of K. In both I and K, three layers of cells (stippled, separated by bold lines) can be discerned at the apex. All of our observations indicate that these three layers will give rise to the SAM. These three layers are typically bounded below by a characteristic semicircular set of walls which is derived from the boundary that separates the descendants of the upper hemisphere from those of the lower. The lateral boundaries of this presumptive SAM are uncertain. We have stippled the three to four most central cells in each layer that span the apex. (M) Fully formed embryo. (N) Tracing of M. Cotyledons have bent 180° and have reached the root end of the embryo. Cells at the shoot apex have divided causing the shoot apex to broaden and resulting in the formation of a histologically detectable SAM. Cells in the epidermal (ed) and upper hypodermal layer (1) have divided anticlinally. Cells in the lower hypodermal layer (2) have divided more irregularly. In this figure, both anticlinal and oblique divisions can be observed in the lower hypodermal layer. The SAM at this stage is roughly seven cells wide. (O) Maturing embryo. (P) Tracing of O. The embryo is accumulating storage substances but has not yet dried down. The SAM now clearly appears as a bulge at the shoot apex. Storage substances appear to be excluded from the SAM. Scale bar, 20 μm.

When viewed with DIC optics, intracellular bodies can be seen throughout most of the wild-type embryo. These intracellular bodies are excluded from the apparent SAM precursor cells in the folding cotyledon stage embryo (Fig. 5). The only structures recognizable in the cells at the apex correspond to nuclei and nucleoli in the stained sections. The distribution of these intracellular bodies is similar to that seen for storage granules in the end-stage embryo and may reflect an early stage in their formation not detectable using methylene blue staining and bright-field optics. The *stm*

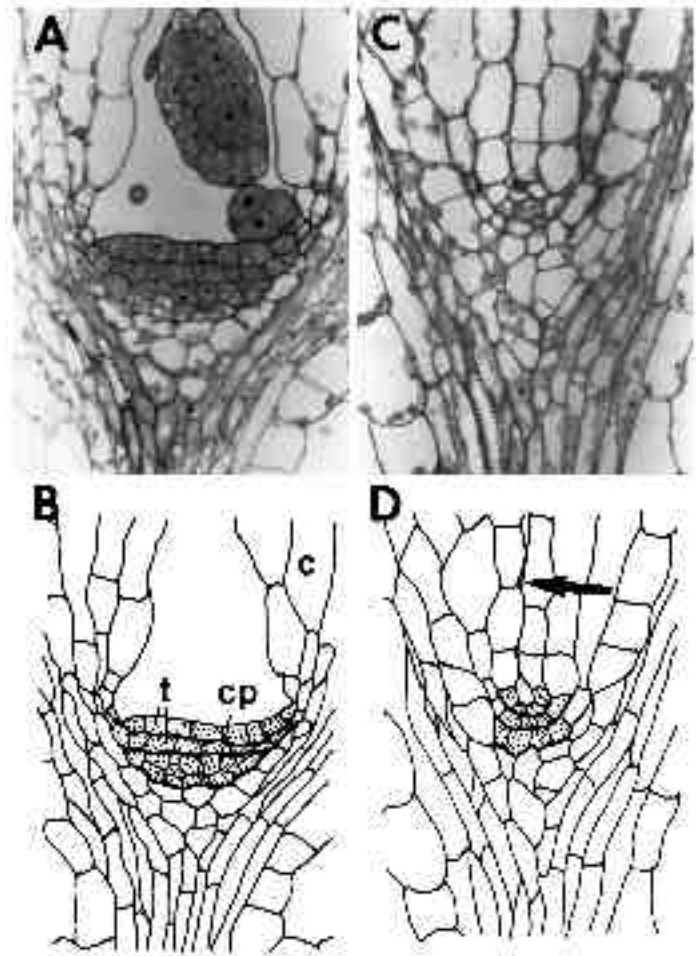


Fig. 2. Median longitudinal sections through wild-type and homozygous *shoot meristemless-1* shoot apices. (A) Wild-type shoot apical meristem, 2 days postgermination. Meristematic cells are densely cytoplasmic and consequently stain more intensely. Note the two-layered tunica that overlies the less well organized corpus. (B) Tracing of A. Stippling indicates those densely cytoplasmic cells that make up the shoot apical meristem. Bold lines indicate boundaries between tunica layers and corpus. c, cotyledon; t, tunica; cp, corpus. (C) Apical region of *shoot meristemless-1* seedling, 2 days postgermination. Meristematic activity is lacking as judged by the absence of densely cytoplasmic cells. (D) Tracing of C. In the absence of a SAM the bases of the cotyledons are closely juxtaposed, arrow indicates point where they meet. Stippling indicates those cells that are in the position normally occupied by the shoot apical meristem. Bold lines delineate the three layers of cells present at the shoot apex in shoot meristemless seedlings. Scale bar, 20 μm.

mutant embryo in contrast has fewer cells at the apex that show exclusion of these bodies (Fig. 5).

In addition, to investigate the possibility that the SAM is merely dying in the *stm* mutant embryos, developing embryos were examined for the presence of dying cells at the shoot apex. Greater than 50 torpedo stage embryos and 50 bending cotyledon stage embryos were examined. (Since median longitudinal sections were not necessary to score the presence of dying cells, more embryos could be examined. For all of these, serial sections of the apex were examined.)



Fig. 3. Homozygous *shoot meristemless-1* and wild-type seedlings, 4 days postgermination. The seedling homozygous for the *shoot meristemless-1* mutation (left) lacks leaves, indicating the lack of a shoot apical meristem. In the wild-type seedling (right) the first true leaves (arrow) are visible at the juncture of the cotyledons (embryonic leaves).

Cell death might be expected to result in the loss of the cytoplasmic sac, leaving only the cell walls. However, all cells at the embryonic apex remained densely cytoplasmic (as judged by their staining with methylene blue) and were often seen to retain intact nuclei and nucleoli (DIC optics).

The analysis of torpedo stage and bending cotyledon stage self-progeny embryos of *stm/+* parents supports the hypothesis that *stm-1* blocks SAM development at or just after the torpedo stage of embryogenesis. This block could be due to an inability of the presumptive SAM cells to divide or it

Table 1. Regeneration of shoots from wild-type and *shoot meristemless* roots

Phenotype	No. of roots cultured	No. of normal shoots regenerated	No. of abnormal* leaves and shoots regenerated
wild-type	20	15	88
<i>shoot meristemless</i>	66	0	44

*We use the term abnormal leaves to describe determinate organs. They are generally flattened but occasionally cylindrical. Abnormal shoots were defined as such if more than one leaflike structure emanated from the same position on the callus.

could reflect a failure of the three layered torpedo apex to undergo the transitions in cell division patterns that are presumably necessary to form a SAM.

At about 14 days of age, *stm* seedlings exhibit an additional phenotype. The hypocotyl just below the cotyledons swells and a mass of leaves bursts from this region (Fig. 6). Additional leaves may burst from the petioles of these leaves. However, a shoot with organized phyllotaxis does not form and the leaves are often abnormal in shape. Since this phenotype develops rather late in the life of these seedlings, it is likely that this is a secondary effect due to the lack, in these mutants, of inhibition of cell growth and proliferation normally provided by a functional SAM.

Adventitious shoot formation by *shoot meristemless* tissue

To ask whether *stm-1* also affects adventitious shoot formation, root explants from *stm-1* seedlings were grown in culture under conditions that promote shoot regeneration (Table 1). Wild-type and *stm-1* explants both formed callus when placed on a callus inducing medium, and both tissues produced green nodules when transferred to shoot-inducing

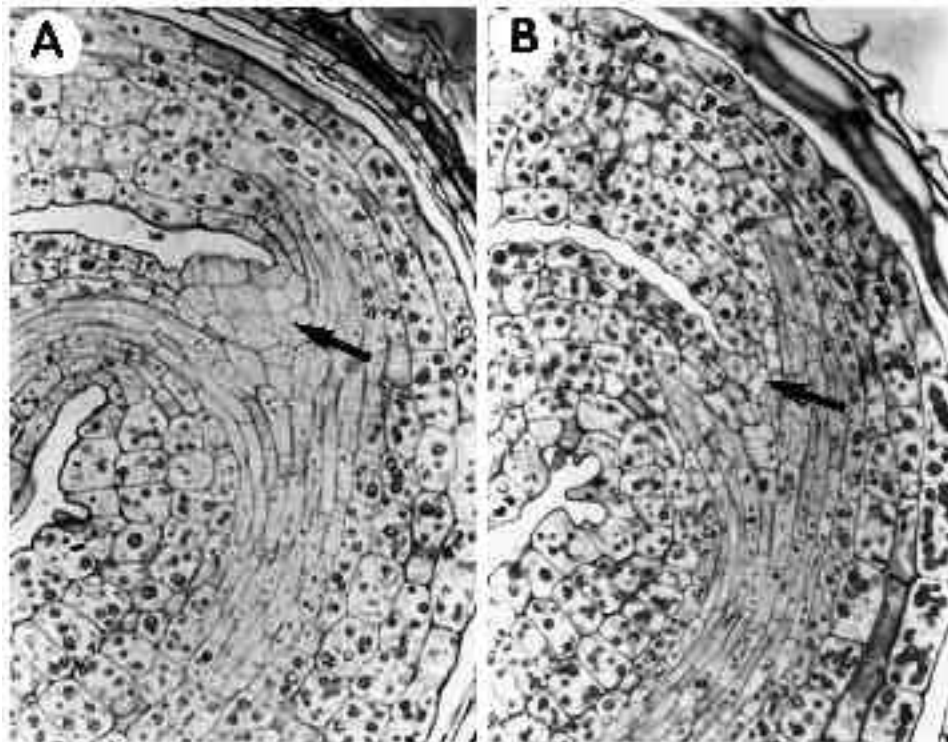


Fig. 4. Median longitudinal sections through wild-type and *shoot meristemless-1* embryos. The wild-type and *shoot meristemless* embryos shown were siblings developing in the same silique. (A) Wild-type fully-formed embryo. SAM has formed at bases of cotyledons (arrow). Note the exclusion of storage granules from the SAM and provasculture. (B) Mature stage *shoot meristemless* embryo. No SAM is present. Bases of cotyledons meet at an acute angle (arrow). Storage granules are inappropriately present in the apical region. Longitudinal sections of a total of 17 self-progeny embryos of a *stm-1/+* parent were examined. 3 of these lacked a SAM. Scale bar, 20 μm .

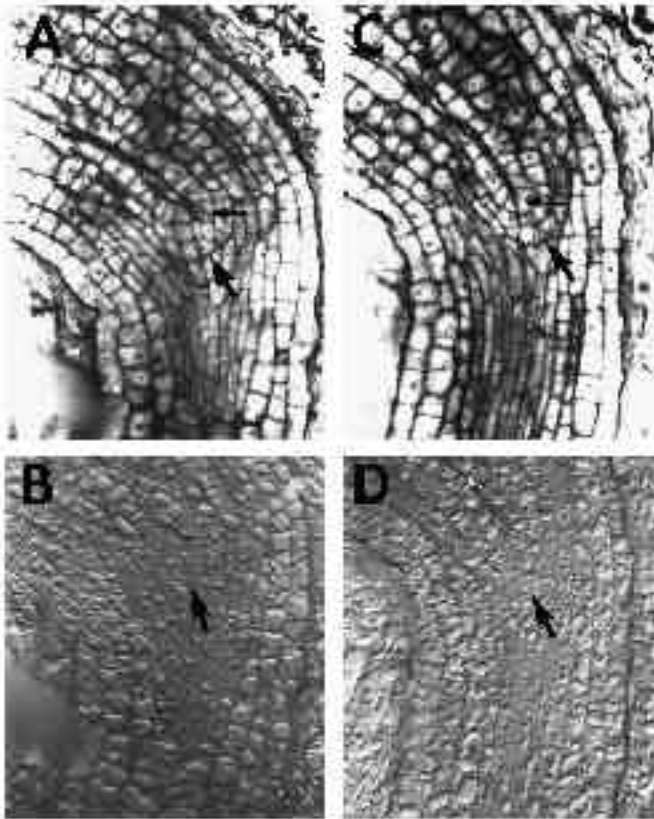


Fig. 5. Wild-type and *stm* bending cotyledon stage embryos. The large arrows point to the boundary subtending the three cell layers that form the SAM in the wild-type embryo. (A) Wild-type embryo. Note that the fold formed by the upper cotyledon as it bends over the embryo (small arrow) is placed off center with respect to the long axis of the hypocotyl, just to the right of the forming SAM. As the region at the apex broadens, the radius of curvature of the walls separating the three layers forming the SAM decreases relative to the torpedo stage embryo. (B) Same section as in A viewed with DIC optics. Large intracellular bodies are seen in most cells of the developing embryo. The cells in the three layers that will form the SAM lack these. (C) *stm* mutant embryo. Fold formed by upper cotyledon as it bends over embryo is at center of long axis of hypocotyl (arrow). The walls that separate the three apical layers remain acutely curved as in the torpedo stage embryo. (D) Same section as in C viewed with DIC optics. Fewer, if any cells at the apex show the exclusion of the large intracellular bodies.

medium. In wild-type cultures a proportion of these nodules subsequently formed normal flowering shoots as well as abnormal shoots and leaves. *stm-1* root explants produced only abnormal leaves or shoots, and overall gave rise to fewer such structures than did wild-type tissue (0.7 versus 4.4 per root). (In three cases, *stm-1* explants formed shoots that had a near normal arrangement of leaves; however, none of these three 'shoots' produced an inflorescence.) Thus, *stm-1* interferes both with the initiation of the SAM during embryogenesis, as well as with shoot initiation in culture. If the *shoot meristemless-1* mutation is a loss-of-function mutation, as suggested by its recessive character, then it defines a locus that is required for SAM initiation both embryonically and postembryonically.

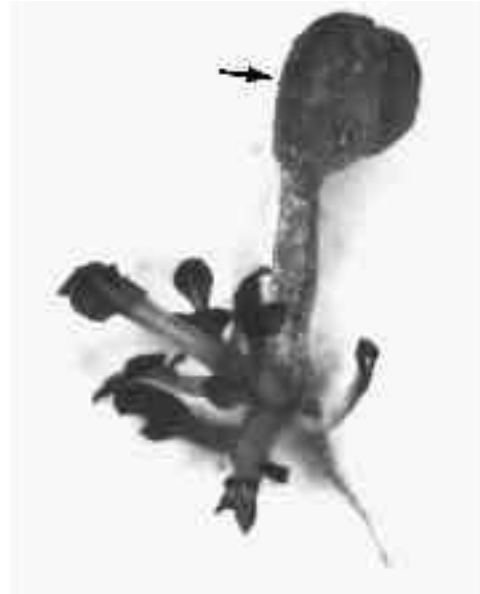


Fig. 6. Phenotype seen in older *stm-1* seedlings. The region of the hypocotyl just below the cotyledons (arrow) swells. Ultimately many, often abnormally shaped, leaves burst from within the hypocotyl.

The phenotype of seedlings homozygous for *rootless* and *shoot meristemless*

We have isolated mutants whose principle defect is the lack of a root apical meristem (our unpublished observations). In addition, these *rootless* (*rtl*) mutations have pleiotropic effects on other portions of the embryo. Among these effects is the lack of a SAM in a fraction of *rootless* embryos. The *rootless* mutants described here are similar in phenotype to *monopteros* mutants described by Mayer et al. (1991) and may be allelic with these.

To further delineate the logic of gene interaction during embryogenesis, double mutants homozygous for both *shoot meristemless* and *rootless* were generated. Plants homozygous for both mutations were constructed by allowing plants heterozygous for both *stm-1* and *rtl-2* to self-fertilize. Three types of mutant seedlings were observed in these families: seedlings lacking a root apical meristem, seedlings lacking a SAM, and seedlings lacking both of these structures. No novel mutant phenotypes were observed, nor was there a significant difference in seed viability in double mutant families relative to families segregating only one of these mutations. Unfortunately, it was impossible to ascertain the genotype of a seedling that lacked both a root apical meristem and a SAM because some *rtl* seedlings lack a SAM even in the absence of the *stm-1* mutation. However, it is likely that this mutant class contained seedlings homozygous for both *rtl-2* and *stm-1* because the frequency of seedlings lacking both a SAM and a root apical meristem was significantly higher (P 0.05; z-test) in families in which both mutations were segregating than in families in which only *rtl-2* was segregating (Table 2). Fourteen percent more root and shoot meristemless plants were found in the self-progeny of $+/rtl-2;+/stm-1$ parents than in the progeny of $+/rtl-2$ parents. (The expected increase if the *shoot meristemless rootless* double mutant phenotype is additive is

Table 2. shoot meristemless rootless double mutant phenotype

Genotype of parent	% shoot meristemless seedlings	% rootless seedlings	% rootless seedlings that are also shoot meristemless
<i>rtl-2/+; +/+</i>	0 (0/1188)	23 (272/1188)	25 (60/237)
<i>rtl-2/+; stm-1/+</i>	22 (263/1178)	23 (267†/1178)	39 (111/284)*

*Not significantly different from the value expected (43.8%) if the phenotype of *shoot meristemless* and *rootless* double mutants is additive ($\chi^2=2.4$).

†This value does not equal 284 as the percentage of *rtl* seedlings was not determined for the self-progeny of one parent.

18.8%.) We conclude that the phenotype of homozygous *stm-1; rtl-2* double mutants is simply the superimposition of the individual phenotypes conferred by these mutations.

DISCUSSION

The primary shoot apical meristem in *Arabidopsis* is initiated during embryogenesis. Additional shoot apical meristems are formed postembryonically - either in the axils of leaves or adventitiously from root explants cultured in vitro. The *stm-1* mutation prevents the formation both of the primary SAM during embryogenesis and of adventitious SAMs. Since *stm-1* mutants never form a normally organized shoot structure, we were not able to assess whether *stm* mutations also prevent the formation of lateral shoot apical meristems in the axils of leaves.

The recessive nature of the *stm-1* mutation suggests that this is a loss-of-function mutation (although not necessarily a null mutation). Thus, our working hypothesis is that the wild-type *SHOOT MERISTEMLESS* gene product is required for SAM initiation, both embryonically and postembryonically. Key regulatory gene products in other systems have been shown to control both the initiation and the subsequent maintenance of developmental decisions (e.g. the *Sex lethal* gene in *Drosophila* sex determination (Cline, 1984) and the *c1* gene in lambda lysogeny (Meyer et al., 1975). By analogy, the *STM* gene product may be necessary not only for the initiation of the shoot meristem but also for its maintenance.

The *stm* defect is specific to the SAM: *stm* mutants show completely normal root growth and development. Aside from demonstrating that the *stm-1* mutation does not affect cell growth or viability, this observation also suggests that the initiation of shoot and root meristems occurs by fundamentally different mechanisms. While these structures are likely to share many gene products (e.g. cyclins), genes such as *SHOOT MERISTEMLESS* are likely to function solely in shoot meristem development. Although other mutations have been isolated that prevent the development of the meristems in the embryo (*rootless* and *topless*: our unpublished observations; *monopteros*, *gnom* and *gurke*: Mayer et al., 1991), thus far *stm-1* is the only mutation that affects only one of the two meristems and does not have other pleiotropic effects on the embryo.

It is important to note that the *stm-1* mutation obliterates the SAM without affecting the ability of the embryo to adopt

other shoot fates. Despite the fact that shoot meristemless mutants lack a shoot apical meristem, they are able to produce structures that have shoot characteristics such as cotyledons (in the embryo) and leaves (adventitiously from the hypocotyl or from root tissue in culture). Thus, the *stm-1* mutation uncouples SAM fate from other shoot fates. This suggests a model in which a developmental control that specifies shoot fate occurs prior to the action of gene products that specify a variety of 'subfates' such as those responsible for initiating SAMs or leaves. Mutations such as *topless* or *gurke* which result in lack of both cotyledons and a SAM may define genes whose products are required for specifying shoot fate in a general way, whereas the *STM* locus may be required only for the development of the SAM.

The shoot apical meristem in wild-type *Arabidopsis* is first apparent during embryonic development as the cotyledons fold over the embryo. Just prior to this, in the torpedo stage embryo, the presumptive SAM consists of three layers of cells roughly four cells in diameter. Although these three layers are direct precursors of the two-layered tunica (the epidermal and upper hypodermal layers of the presumptive SAM) and of the underlying corpus (the lower hypodermal layer), they do not yet show the tunica/corpus organization characteristic of the SAM. This organization becomes apparent at the bending cotyledon stage of development when anticlinal divisions in the upper two layers and a mixture of oblique, periclinal and anticlinal divisions in the lowest layer of the presumptive SAM occur. These cell divisions signal the first point in development at which a SAM is histologically detectable. Thus, the initiation of the SAM is a late event in embryogenesis, occurring after all other major tissue systems of the embryo have appeared - i.e., the root apical meristem, the hypocotyl and the cotyledons.

The analysis of *stm* mutants at various developmental stages argues strongly that these mutants are blocked at the torpedo stage of SAM development. Examination of the terminal phenotype of *stm* mutant seedlings shows that the cells at the position that the SAM would normally occupy are present in these mutants in a three-layered configuration roughly four cells wide. This is the same configuration of cells seen at the presumptive SAM in the torpedo stage embryo. Moreover, *stm* mutant embryos at the bending cotyledon stage (the stage at which a SAM would normally be forming) show a phenotype entirely consistent with a block in SAM development at the torpedo stage: the three cell layers at the apex fail to broaden and remain essentially in the same configuration as in the preceding torpedo stage. A block in SAM development at the torpedo stage could result either from the inability of SAM precursor cells to divide or from a failure of the three cell layers to adopt the appropriate division patterns necessary to form a SAM.

The failure of *stm* mutants to initiate a SAM and the fact that these mutants are blocked just prior to the point at which the SAM becomes histologically distinct is consistent with the hypothesis that the initiation of the SAM coincides with its histological differentiation. This is in contrast to an alternative model for the initiation of the SAM, which assumes that the entire apical half of the globular stage embryo is the SAM. According to this second hypothesis, the first structures produced by the SAM are cotyledons.

An intriguing possibility suggested by our results is that an organized shoot apical meristem may not be required to form leaves. This is based on the observations that cotyledons - which are very leaflike - form before a SAM does and that *stm* mutant tissue is able to form leaves (albeit abnormal) both in culture and in situ from the hypocotyl.

How similar is the initiation of the SAM in the *Arabidopsis* embryo to the initiation of shoot meristems in other angiosperms? In torpedo stage embryos of both *Stellaria media* and *Capsella bursa pastoris*, three layers of cells immediately overlie the provascular tissue at the site of the presumptive SAM, just as in *Arabidopsis* (Ramji, 1975, 1976; Souèges, 1914). The organization of cells in these species is similar if not identical to that of *Arabidopsis*. However, whereas the patterns of cell divisions that give rise to this three-layered intermediate in *Capsella* are identical to that in the *Arabidopsis* embryo, the pattern of cell divisions that give rise to this common intermediate are very different in *Stellaria*. Subsequent steps in meristem formation may vary as well (Ramji, 1976). The common feature of all three species is that the SAM (as defined by a structure that exhibits a tunica/corpus arrangement of cells) does not appear until well after the cotyledons are initiated.

We would like to acknowledge members of the Poethig laboratory for helpful discussion and especially Abby Telfer and Matthew Evans for critical reading of the manuscript. Deverie Pierce provided excellent technical assistance. This work was supported by grant no. 91-37304-5977 from the USDA. M. K. B. was the recipient of Plant Postdoctoral Fellowship no. DIR-8906070 from the NSF. This is paper no. 3381 from the Laboratory of Genetics.

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(Accepted 17 August 1993)