for the initiation and development of ENSO. They may also improve our understanding of upper-ocean processes, air-sea exchanges, and the global atmosphere.

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EMF, an *Arabidopsis* Gene Required for Vegetative Shoot Development

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In higher plants, the transition from the vegetative to the reproductive state in the shoot meristem initiates flowering. To study this floral transition, a constitutively flowering mutant of *Arabidopsis thaliana* ecotype *columbia, embryonic flower* (*emf*), was characterized. No vegetative shoots were produced from *emf* embryos or calli; the shoot apical meristems (SAMs) in the *emf* embryos were altered compared to wild-type SAMs. The mutant SAMs enlarged precociously and produced inflorescence meristems upon germination. These results suggest that the dominant, wild-type allele *EMF* is required for the vegetative state of the SAM. In the absence of *EMF* function, the mutant embryo assumes the reproductive state.

The shoot apical meristem (SAM) is the origin of the shoot system; its cellular products differentiate as vegetative or reproductive organs. The SAM is formed during embryogenesis and can be identified by its terminal position and its tunica corpus cellular arrangements (*1*). However, little is known about how it is formed and how it functions. In *Arabidopsis* (*2*, *3*), specific genes regulate meristem ontogeny (*4*) and fate determination (*5*–*7*). Here, we describe a mutant that identifies a gene, *EMF*, involved in the switch between the vegetative and the reproductive meristem.

Four independent *emf* mutants were recovered from ethyl methane sulfonate (EMS)– and gamma-ray–mutagenized *Arabidopsis* seeds by screening for individual plants that segregated 25% offspring with abnormal phenotypes (Fig. 1); the data presented are based on the characterization of the first mutant recovered from the EMS-mutagenized *Arabidopsis* seeds. The wild-type *Arabidopsis* is a semirosette plant (*8*). There is no internode elongation in the vegetative shoot until the onset of flowering. Whereas the heterozygous plants were indistinguishable from the wild-type plants, the homozygous *emf* plants did not produce a normal rosette upon germination but rather produced several cauline leaves and multiple floral buds (Fig. 1A) that later set fruits (Fig. 1B). Flowers of *emf* plants were

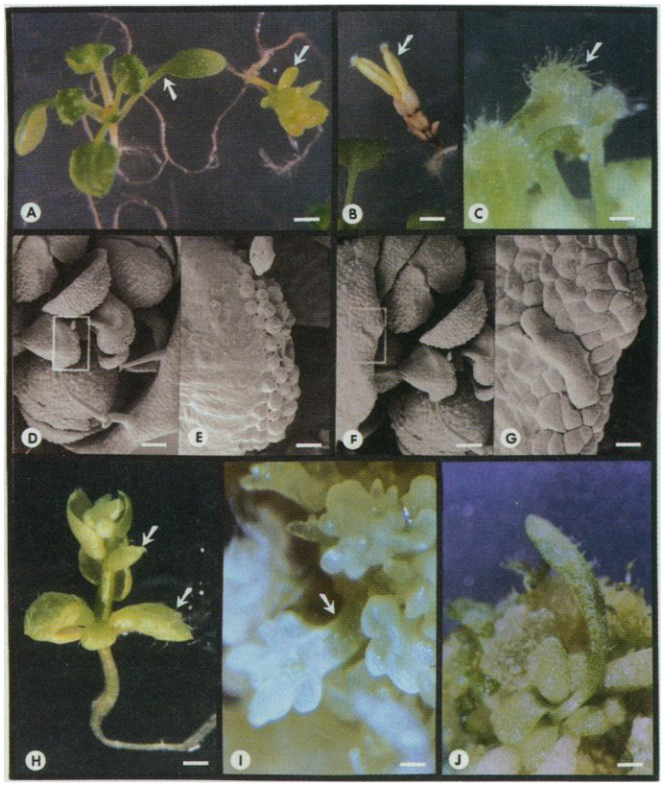

Fig. 1. (**A**) Normal (*EMF*, left) and mutant (*emf*, right) plants 18 days after germination. Seeds from a heterozygous plant were sterilized and vernalized for 3 days and then grown under 9 hours of light at 21°C on agar plates that contained half-strength Murashige and Skoog (MS) medium (*11*) without plant growth regulators. The normal plant is at the rosette stage with two cotyledons and four leaves; all have long petioles. The mutant has petioleless cotyledons and several sessile leaves and floral buds. Arrows point at the cotyledons. Bar = 1.0 mm. (**B**) Normal (bottom) and mutant (top) plants 6 weeks after germination under 9 hours of light per day. The *emf* mutant shows two fruits or siliques. Arrow points at the silique. Part of a normal plant (shown at the bottom) has rosette leaves with long petioles but no flowers. Bar = 1.1 mm. (**C**) Regenerating *emf* tissue on shoot-inducing medium (SIM), showing structures that resemble pistils. Root explants of *emf* seedlings were placed on callus-inducing medium [B5 medium (*11*) that contained 2,4-D (0.5 mg/liter) and kinetin (0.05 mg/liter)] for 4 weeks. Calli were transferred onto SIM [B5 medium + indole acetic acid (0.15 mg/liter)] and 2-isopentenyl adenine (5 mg/liter) for 6 weeks. Arrow points at papillae-like structures. Bar = 0.2 mm. (**D**) Scanning electron micrograph (SEM) of a mutant flower. Bar = 60.0 μm. The boxed area, enlarged in (**E**), shows the papillae on the pistil. Bar = 11.6 μm. (**F**) SEM of a mutant, showing the sepal-like structure. Bar = 59.9 μm. The boxed area, enlarged in (**G**), shows an elongated cell, characteristic of sepal cells. Bar = 11.8 μm. (**H**) A 20-day-old *emf* mutant plant showing inflorescence, two petioleless cotyledons, a small stem, cauline leaves, and several floral buds. Arrows point at the cauline leaves. Bar = 0.48 mm. (**I**) Regenerating *emf* tissue on SIM, showing flower-like structures. Arrow points at a small stem resembling the pedicel upon which whorled organs are situated. Bar = 0.2 mm. (**J**) Regenerating normal tissue on SIM, showing the rosette-like shoot. Bar = 0.61 mm.

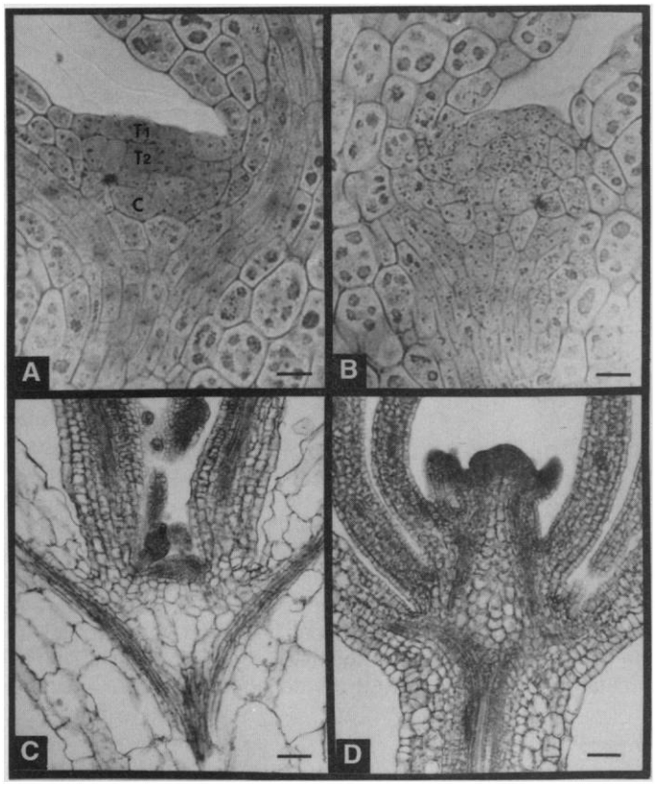

Fig. 2. Differences in the SAM structures of wild-type and mutant embryos. (**A**) Median longitudinal section of a normal SAM in mature embryos, showing the tunica corpus zonation, the outer tunica (T1), the inner tunica (T2), and the corpus (C) layer. Seeds from a heterozygous plant were autoclaved for 5 min to allow penetration of the fixative and embedding material through the dry embryo. The seed coats were removed, and the embryos were fixed in 4% glutaldehyde for 16 hours. Fifty embryos were embedded in JB-4 plus embedding medium (Polysciences, Warrington, Pennsylvania). Longitudinal serial sections of 3 μm were made with the use of a retracting microtome (Microm HM350, Heidelberg, Germany). Sections were stained with 0.05% toluidine blue and examined under a microscope to identify the median section. Bar = 4.2 μm. (**B**) Median longitudinal section of an abnormal SAM in a seed from a heterozygous *emf* mother plant. The SAM is elevated; the cells display no clear tunica corpus organization. Bar = 4.0 μm. (**C**) Median longitudinal section of a 10-day-old wild-type seedling grown under 9 hours of light per day. Seedlings were fixed in Formalin acetic acid and embedded in paraffin. Longitudinal sections of 8 μm were prepared and stained with Orange-G. Bar = 17.1 μm. (**D**) Median longitudinal section of a 9-day-old *emf* mutant grown under 9 hours of light per day, showing the inflorescence meristem. Bar = 17.1 μm.

Table 1. Effects of the presence and absence of cotyledons and embryonic roots on SAM development in normal *Arabidopsis* and *emf* plants. Seeds from a heterozygous plant were sterilized and soaked in water for 4 hours. The seed coats were then removed, and the embryos were dissected under sterile conditions. Because the embryos were still desiccated, the cotyledons broke off readily. Embryonic roots were cut off about 200 μm from the cotyledon base. We prepared the isolated shoot apexes, about 200 μm in length, by breaking off the cotyledons at the base first and then cutting off the root, leaving the SAM attached to an embryonic axis of 200 μm. The intact and embryonic pieces were germinated on half-strength MS medium under 9 hours of light per day at 21°C. Cultured on hormone-free medium, the embryonic pieces depended on endogenous hormones for their growth. The germinating −cot embryos produced shoots from the SAM but did not produce any cotyledons except for a small stub of petiole that grew occasionally. The −root embryos generally had their hypocotyls removed also but could produce adventitious roots. Those that grew and produced rosette leaves with petioles were scored as normal; they could be either wild-type or heterozygotes. Those that produced sessile leaves and pistils by 30 days were scored as mutant. Mutants generally grow slower; their survival rate can be improved if one provides the embryonic pieces with exogenous growth regulators. Given a pulse (2 days) of 2,4-D (0.5 mg/liter) and kinetin (0.05 mg/liter), more than 50% of the wild-type isolated apexes germinated and produced new leaves in less than 1 week. Results obtained from growth regulator–pulsed shoot apexes (*12*) confirm the data presented in the table, which is representative of three separate experiments.

Embryonic organs cultured	Plants (*n*)		
	Normal (rosette)	Mutant (inflorescence)	No growth
Intact embryo	30	9	1
−cot embryo	27	7	12
−root embryo	34	9	20
−cot,−root embryo (isolated apex)	17	4	34

usually abnormal and incomplete. Every plant produced pistils (Fig. 1, D and E), but only some mutants contained anthers, which did not produce pollen. Petals were rarely found in the mutants; sepals were difficult to distinguish from cauline leaves except for the presence of elongated cells and the absence of trichomes (Fig. 1, F and G). The pistils of the mutants contained ovules, but no viable seeds were produced, and root growth was normal and vigorous.

Early defects were apparent at the formation of the vegetative shoot in the mutants. Although several leaves formed before the appearance of floral buds, these leaves appeared to be cauline, not rosette, leaves because they were sessile—that is, they had no petioles and were situated on a stem (Fig. 1H). In some *emf* plants, the cauline leaves had a flower or a secondary floral branch in the axil. Thus, *emf* mutants appear to germinate into an inflorescence shoot directly, bypassing the vegetative shoot.

Arabidopsis is a facultative long-day plant in which flower initiation is accelerated by long photoperiods. However, the *emf* phenotype was expressed in both mutant and wild-type plants grown under long- (16 hours of light) or short-day conditions (9 hours of light). The early flowering phenotype of the mutant embryo was not affected by the growth conditions of the mother plant. When grown in total darkness, cotyledons did not expand in either the mutant or wild type although seedlings did germinate and segregated in a 3:1 ratio as long- and short-hypocotyl plants. When switched to light, the short-hypocotyl plants were confirmed to be the mutants by their petioleless cotyledons and their inability to produce rosettes. Within 3 days of germination and under either short- or long-day conditions, mutant seedlings could be morphologically distinguished from normal plants by cotyledon shape and hypocotyl length. Whereas the wild-type and heterozygous plants had round cotyledons with long petioles, the mutants produced oval-shaped, petioleless cotyledons (Fig. 1A). In 7 days, the hypocotyls of the mutants reached on average 1.0 ± 0.043 mm, about half of that of the wild type, which was 1.89 ± 0.062 mm.

Although the gross morphology of the mutant embryo is always indistinguishable from that of the wild type, the structures of their SAMs differ in shape (Fig. 2). We sectioned the embryos and examined the median longitudinal sections of the SAMs. Out of 45 mature embryos from a selfed heterozygous plant, 31 had flat SAMs that had two tunica layers and one corpus layer (Fig. 2A) and 11 had convex SAMs (Fig. 2B). No good median sections were found in three embryos. Many of the tunica layers in the convex SAMs contained cells of

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irregular shape and size (Fig. 2B). The ratio of 31 to 11 suggests that the embryos with convex SAMs are the homozygous recessive mutants. In 33 wt embryos, no convex SAMs were found.

The altered SAM in mutant embryos and seedlings could result from precocious development of the inflorescence meristem. We found that the 3-day-old mutant seedlings (with sessile, oval cotyledons and short hypocotyls) had slightly elevated meristems, whereas normal seedlings (with round cotyledons with petioles) had flat meristems. After 9 days, the SAMs of the *emf* mutants were enlarged, and some had already developed into an inflorescence meristem (Fig. 2D) that later formed floral meristems. The development of the inflorescence and floral meristems in the mutant is similar to their development in wild-type plants (9). The SAM of a normal seedling at the same stage remained small and flat (Fig. 2C). Moreover, cells in the mutant cotyledons and hypocotyls were much smaller than those in the wild type (Fig. 2, C and D). In normal plants, both leaf and cell size of cauline leaves are smaller than those of rosette leaves. The small cotyledons and short hypocotyls in the mutants may result from reduced cell expansion.

The embryo of the *emf* mutant produced either a reproductive SAM directly or an extremely short-lived vegetative meristem that was converted to a reproductive meristem before germination. Calli were initiated from *emf* and normal plants and transferred to shoot-inducing medium to regenerate shoots. However, only wild-type calli produced rosette shoots (Fig. 1J). Various floral structures, including pistils (Fig. 1, C and I) and occasionally an inflorescence, arose directly from mutant calli. The inability of the *emf* mutant to produce vegetative shoots in the embryo and the callus supports the notion of a constitutive flowering mutant.

Surgical experiments of flowering mutants in pea revealed that some flowering genes act in the shoot apex and other genes act in the cotyledons and leaves by controlling the production of a floral inhibitor that is transmitted to the SAM to suppress flowering (*10*). To study the site of gene action of the wild-type and mutant alleles (*EMF* and *emf*, respectively), we cut off parts of the semidry embryos under sterile conditions and germinated the fragments on half-strength Murashige and Skoog medium (*11*) with sugar. If the cotyledons or the embryonic root were required for meristem function, the removal of these organs would affect the type of shoot produced by the SAM. In all cases, embryos without root (−root) and without cotyledons (−cot) and isolated apexes devoid of cotyledons and the root (−cot,−root) produced vegetative or reproductive shoots in culture in amounts similar to those produced by intact embryos (Table 1). We conclude that there is no floral inhibitor in normal *Arabidopsis* cotyledons and that the expression of the mutant phenotype is independent of the presence of cotyledons. Thus, the *EMF* gene acts in the shoot apex, which functions autonomously to produce rosettes or inflorescences.

If the recessive allele *emf* is a loss-of-function mutation, the function of the dominant allele *EMF* would be to activate the vegetative state or to suppress the reproductive state of the shoot apex. In the absence of the *EMF* gene product, the mutant changes its growth pattern to form an inflorescence. We propose that young *Arabidopsis* plants normally produce *EMF* products. As the plants age, the amount of the *EMF* gene product diminishes, and its disappearance may be facilitated by long-day conditions. Because the *EMF* gene product converts the SAM to the vegetative state in wt plants, the reproductive state would be the default state of the SAM in these plants.

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A Homoeotic Mutant of *Arabidopsis thaliana* with Leafy Cotyledons

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Cotyledons are specialized leaves produced during plant embryogenesis. Cotyledons and leaves typically differ in morphology, ultrastructure, and patterns of gene expression. The *leafy cotyledon* (*lec*) mutant of *Arabidopsis thaliana* fails to maintain this distinction between embryonic and vegetative patterns of plant development. Mutant embryos are phenotypically abnormal, occasionally viviparous, and intolerant of desiccation. Mutant cotyledons produce trichomes characteristic of leaves, lack embryo-specific protein bodies, and exhibit a vascular pattern intermediate between that of leaves and cotyledons. These results suggest that *lec* cotyledons are partially transformed into leaves and that the wild-type gene (*LEC*) functions to activate a wide range of embryo-specific pathways in higher plants.

Embryo development in seed plants involves two fundamental processes: (i) morphogenesis and the establishment of root and shoot apical meristems and (ii) preparation for desiccation, dormancy, and germination. The genetic basis of these developmental programs has been explored in part through the isolation and characterization of embryonic mutants (*1*). Emphasis has been placed on embryonic lethals and defectives of maize (*2*) and *Arabidopsis* (*3*). Embryonic pattern mutants with defects in basic plant organization have also been identified (*4*). The *leafy cotyledon* (*lec*) mutant of *Arabidopsis* is a homoeotic mutant with defects in embryonic maturation and the normal distinction between embryonic cotyledons and post-embryonic leaves. Homoeotic mutants have played an important role in the genetic dissection of development in *Drosophila melanogaster* (*5*) and floral development in angiosperms (*6*). Although a wide range of homoeotic conversions has been observed in plants (*7*), mutations that transform embryonic cotyledons into foliage leaves have not been previously reported. The mutant phenotype described here suggests that a single regulatory gene may control many of the differences between leaves and cotyledons in higher plants.

Seed development in *Arabidopsis* culminates with the accumulation of storage pro-

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