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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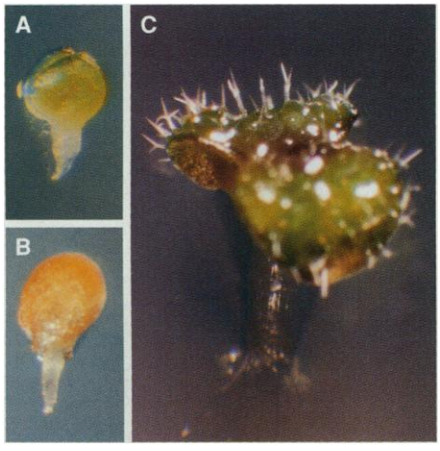

Fig. 1. Phenotype of *leafy cotyledon*. (**A**) Immature embryo with rounded cotyledons and a short hypocotyl. (**B**) Viviparous seed with protruding root at maturity. (**C**) Rescued mutant seedling with trichomes on cotyledons.

teins and lipids, the acquisition of desiccation tolerance, the loss of chlorophyll, and seed dormancy. The *lec* mutant fails to exhibit these characteristic features of embryonic maturation. This mutant was originally identified by screening immature siliques from transgenic plants produced by *Agrobacterium tumefaciens*–mediated seed transformation (*8*). Subsequent genetic studies demonstrated that the mutation was not caused by a transferred DNA (T-DNA) insertion (*9*). Heterozygous plants appear normal except for the presence of defective seeds (25%) after self-pollination. Mutant embryos remain green late in development and exhibit a wide range of abnormalities. Mutant cotyledons are rounded, contain unusual protrusions on their adaxial surface, and often accumulate anthocyanin at low temperatures. The hypocotyl is reduced in size and pigmentation (Fig. 1A), and the root apical meristem often becomes active prematurely, occasionally resulting in viviparous seeds (Fig. 1B). Protruding roots are present in approximately 5% of mutant seeds. Roots emerge during embryonic maturation and extend less than 4 mm in length. Mature seeds are intolerant of desiccation and partially deflated and fail to germinate in culture.

These observations initially suggested that mutant embryos were defective in maturation and precociously entered a germination pathway. A similar pattern of development has been described in viviparous mutants of maize defective in abscisic acid (ABA) biosynthesis and response (*10*). None of the related ABA mutants of *Arabidopsis* are viviparous as monogenic lines (*11*). A double mutant (*aba/aba; abi3/abi3*) with reduced ABA content and response during embryogenesis produces seeds that remain green late in development and lack desiccation tolerance (*12*), but these seeds do not exhibit other features characteristic of *lec*. Mapping with visible markers (*13*) has confirmed that *lec* is not allelic to existing ABA mutants of *Arabidopsis*.

The most intriguing feature of *lec* is revealed when mutant embryos are removed from immature seeds and cultured (*14*) before desiccation. The resulting seedlings appear normal except for the presence of trichomes on cotyledons (Fig. 1C). Trichomes are visible within 48 hours of plating and develop from surface protrusions present on cotyledons within the seed. Trichome formation is therefore not induced by contact with the culture medium. Typically, 15 to 20 trichomes are present on the adaxial surface of each cotyledon. Mutant trichomes are often less branched than normal ones. The formation of trichomes on mutant cotyledons is blocked in genetic backgrounds (*gl1; ttg*) that eliminate trichomes on leaves. Mutant seedlings produced in culture develop into plants in soil that appear normal except for the presence of defective seeds (100%) after self-pollination. The expression of *LEC* is therefore not required to complete later stages of the life cycle. Mutant seeds produced by self-pollination of rescued homozygotes resemble those produced by heterozygotes. Pollination of homozygotes with wild-type pollen results in phenotypically normal seeds. This demonstrates that loss of *LEC* function in maternal tissues does not disrupt embryogenesis.

Trichomes in *Arabidopsis* are consistently found on leaves and stems but not on cotyledons of wild-type plants. Mutants with trichomes on cotyledons have not been previously described despite extensive screening of mutagenized populations. Trichomes are not found on wild-type cotyledons after precocious germination of immature embryos. The presence of trichomes on *lec* cotyledons is therefore not simply a consequence of precocious germination. The potential to form trichomes on cotyledons is present in the Brassicaceae family (*15*) but appears to be repressed in *Arabidopsis*. Mutations that remove this inhibition and allow trichomes to form on cotyledons may be lethal because they also result in desiccation intolerance during embryonic maturation.

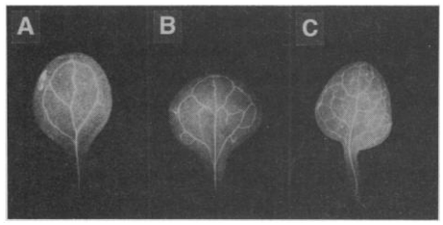

Fig. 3. Patterns of vascularization in cleared leaves and cotyledons from 14-day-old seedlings in culture examined through a dissecting microscope. (**A**) Wild-type cotyledon. (**B**) Mutant cotyledon. (**C**) Wild-type first leaf. These structures were placed in 95% ethanol and cleared in an aqueous solution of lactic acid, phenol, and glycerine.

The ultrastructure of *lec* cotyledons was examined to determine whether homoeosis was limited to epidermal cells. Emphasis was placed on protein and lipid bodies, structures characteristic of embryonic maturation in *Arabidopsis* (*16*). Mutant and wild-type embryos were removed from seeds before desiccation and prepared for electron microscopy (*16*). Wild-type embryos contained many protein and lipid bodies in both the hypocotyl and cotyledons (Fig. 2A). Mutant embryos lacked these embryo-specific markers (Fig. 2B) and exhibited an internal anatomy intermediate between that of leaves and normal cotyledons. Mutant cotyledons also contained numerous plastids in cells near the cotyledon surface.

The extent of transformation of mutant cotyledons into leaves was then examined by comparing the internal patterns of vascularization. Mutant and wild-type leaves and cotyledons were cleared in lactophenol and examined beneath a dissecting microscope. Wild-type cotyledons have a simple pattern of vascularization (Fig. 3A) that divides the cotyledon into several chambers. Although the precise pattern of vascular branches differs between cotyledons, the extent of branching remains relatively constant. Leaves have a more complex pattern of vascularization (Fig. 3C). Mutant cotyledons (Fig. 3B) exhibit a vascular

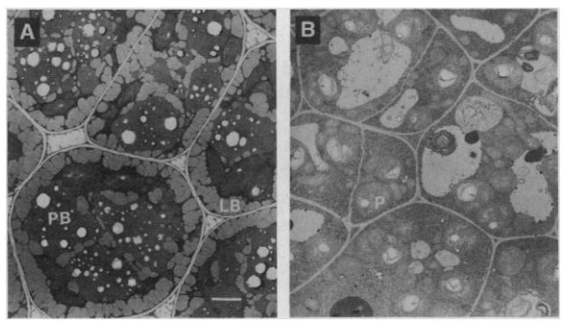

Fig. 2. Ultrastructure of mutant and wild-type cotyledons from immature seeds. (**A**) Wild-type cells with protein bodies (PB) and lipid bodies (LB). (**B**) Mutant cells with numerous plastids (P) but no protein bodies or lipid bodies. Scale bar = 2 μm.

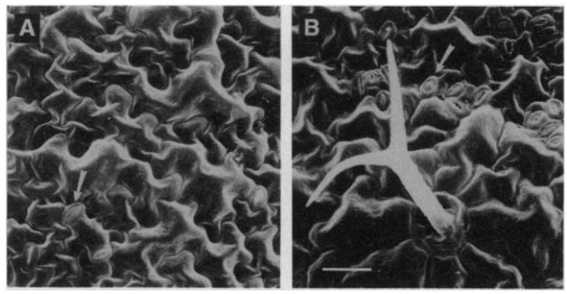

Fig. 4. Scanning electron micrograph of adaxial surface of mutant and wild-type cotyledons from 10-day-old seedlings in culture. (**A**) Wild-type cotyledon with stomata (arrow) but no trichomes. (**B**) Mutant cotyledon with stomata (arrow) and branched trichome. Scale bar = 40 μm.

pattern intermediate between that of leaves and normal cotyledons. These results provide further evidence that the formation of different cell types within mutant cotyledons is disrupted. Stomata were not informative markers because both mutant and wild-type cotyledons contained guard cells after germination (Fig. 4). Mutant cotyledons produced by immature embryos rescued on a minimal medium closely resembled wild-type cotyledons in size and shape. Mutant cotyledons produced on a regeneration medium (*17*) were larger (10 to 20 mm in length) and more closely resembled the shape of wild-type leaves. This dramatic growth of mutant cotyledons in culture has not been observed with other mutants defective in embryo development (*18*).

Several models have been considered to explain the phenotype of this mutant. The first is that *LEC* functions to repress the appearance of leaf traits in cotyledons. One problem with this model is that viable mutants with trichomes on cotyledons have not been identified. Plants segregating for weak *lec* alleles might be expected to produce mutant seeds that survive desiccation and form seedlings with trichomes on cotyledons. The normal appearance of vegetative structures produced by rescued homozygotes is also potentially inconsistent with this model. If *LEC* is a regulatory gene that inhibits the appearance of leaf characteristics in cotyledons, it might be expected to perform a similar function at other stages of the life cycle. There is little evidence that repressors of leaf characteristics play an important role in plant development.

An alternative model is that *lec* is a heterochronic mutant defective in the timing of cell specialization during embryogenesis. Heterochrony and homoeosis are often difficult to distinguish in plants because temporal changes in the shoot apex are preserved as spatial alterations along the stem (*19*). Although *lec* could be viewed as heterochronic to the extent that mutant cotyledons resemble structures produced later in development, there is no evidence that the shoot apex of mutant embryos or rescued mutant plants is altered in developmental timing. Mutant cotyledons appear to form in a normal manner at the heart stage of development. The phenotype of *leafy cotyledon* is therefore more consistent with the classical view of homoeosis in plants (*7*) than with heterochronic shifts observed in mutants of maize (*20*).

The preferred model is that *LEC* functions to activate a wide range of embryo-specific pathways in plants. Loss of gene function disrupts embryonic maturation and returns mutant cotyledons to a basal developmental state. The leafy appearance of mutant cotyledons was unexpected because there was no evidence that cotyledons defective in maturation should be transformed into foliage leaves. However, this observation is consistent with the origin of cotyledons as specialized leaves during plant evolution and the homology of embryonic cotyledons and vegetative leaves. The origin of cotyledons during plant embryogenesis and the precise relationship between leaves and cotyledons in plants have long been subject to interpretation. Leaves are usually produced as lateral outgrowths of an organized shoot apical meristem. Cotyledons are initiated early in embryogenesis, before the shoot apex becomes established, and typically differ from leaves in morphology, ultrastructure, and patterns of gene expression. Cotyledons with unusual patterns of development have been noted in *Streptocarpus*, where one cotyledon enlarges after germination and serves as the principal leaf throughout vegetative development (*21*), and in *Eranthis*, where treatment of immature embryos with cytokinin results in cotyledons that resemble primary leaves (*22*).

The phenotype of *leafy cotyledon* suggests that the difference between leaves and cotyledons in *Arabidopsis* is controlled by a single regulatory gene (*LEC*) expressed only during embryogenesis. This mutant also demonstrates that a single gene controls most essential features of embryonic maturation in *Arabidopsis*. This putative regulatory gene may have facilitated specialization of cotyledons during plant evolution by allowing a wide range of embryo-specific pathways to be controlled by a single regulatory factor. The inhibition of trichomes on cotyledons is part of this regulatory network in *Arabidopsis*.

Molecular studies of plant embryogenesis indicate that a diverse array of transcriptional activators are likely to bind upstream of genes expressed during embryonic maturation. The *LEC* gene product may interact with these transcription factors to ensure proper expression of embryo-specific functions during plant development. A similar but more limited role has been postulated for the *VP1* gene product in maize (*23*). *LEC* may interact with the *VP1* homolog in *Arabidopsis* or regulate its expression early in development. The recent discovery that *ABI3* shares regions of high sequence similarity with *VP1* (*24*) and may perform a related function in *Arabidopsis* is consistent with the view that basic mechanisms of embryonic maturation are conserved among angiosperms and that *LEC* performs a critical function not previously described in plants. Molecular cloning of *LEC* and overexpression of the wild-type gene in transgenic plants could thus lead to enhanced maturation of somatic and zygotic embryos from a variety of plant species.

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Overexpression of a Transporter Gene in a Multidrug-Resistant Human Lung Cancer Cell Line

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The doxorubicin-selected lung cancer cell line H69AR is resistant to many chemotherapeutic agents. However, like most tumor samples from individuals with this disease, it does not overexpress P-glycoprotein, a transmembrane transport protein that is dependent on adenosine triphosphate (ATP) and is associated with multidrug resistance. Complementary DNA (cDNA) clones corresponding to messenger RNAs (mRNAs) overexpressed in H69AR cells were isolated. One cDNA hybridized to an mRNA of 7.8 to 8.2 kilobases that was 100- to 200-fold more expressed in H69AR cells relative to drug-sensitive parental H69 cells. Overexpression was associated with amplification of the cognate gene located on chromosome 16 at band p13.1. Reversion to drug sensitivity was associated with loss of gene amplification and a marked decrease in mRNA expression. The mRNA encodes a member of the ATP-binding cassette transmembrane transporter superfamily.

Small cell lung cancer (SCLC) accounts for 20 to 25% of all lung cancers. It differs from other forms of lung cancer, known collectively as non–small cell lung cancers (NSCLC), because it is initially much more responsive to chemotherapy. Up to 90% of SCLC tumors respond to chemotherapy, but patients almost always relapse with multidrug-resistant disease. The initial response rate of NSCLC tumors is much lower and, for the most part, these tumors display inherent drug resistance. The molecular basis of drug resistance in both SCLC and NSCLC is poorly understood.

Overexpression of the transmembrane transport protein P-glycoprotein has been detected in many multidrug-resistant tumor cell lines and in a variety of tumors from cancer patients with both acquired and inherent drug resistance (*1*). This protein is encoded by the human *MDR1* gene, and in vitro studies have shown that it confers resistance to a range of natural product xenobiotics that are used as chemotherapeutic drugs (*2*). However, despite the widespread occurrence of drug resistance in human lung tumors, overexpression of P-glycoprotein is infrequent, which indicates the existence of alternative resistance mechanisms (*3*, *4*).

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NCI-H69 (H69) is an SCLC cell line that has been used in experimental studies of lung cancer (*5*). We have described a multidrug-resistant variant of this cell line, designated H69AR, which was obtained by stepwise selection in doxorubicin. H69AR displays a drug resistance pattern typical of that associated with increased amounts of P-glycoprotein. However, as with most clinical specimens of SCLC, H69AR does not overexpress this protein or its cognate mRNA (*6*–*8*). Consistent with this observation, major differences in net drug accumulation or efflux do not appear to be part of the resistance phenotype of the H69AR cell line (*8*). Another feature that distinguishes H69AR from cell lines that overexpress P-glycoprotein is the inability of cyclosporin A and several other chemosensitizing agents to reverse doxorubicin resistance in these cells (*7*, *9*).

As part of a search for proteins responsible for the multidrug resistance displayed by H69AR cells, a randomly primed cDNA library was constructed from H69AR mRNA and screened by differential hybridization with total cDNA prepared from H69 and H69AR mRNA (*10*). One of the

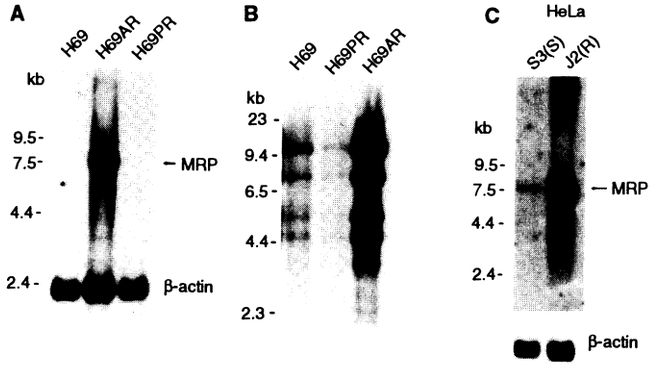

Fig. 1. (**A**) Northern blot of poly(A)$^+$ RNA from H69, H69AR, and H69PR cells hybridized with a 1.8-kb Eco RI cDNA fragment of MRP. The analysis of 1 μg of poly(A)$^+$ RNA from each cell line was carried out with standard procedures (*27*). The autoradiograph shown is a 5-hour exposure at −70°C with intensifying screens. The size of the overexpressed mRNA in H69AR cells, indicated by the arrow, was estimated to be 7.8 to 8.2 kb. Prolonged exposure of the film revealed small amounts of this mRNA in both H69 and H69PR cells. Molecular size standards are shown at the left. (**B**) Southern blot analysis of Eco RI–digested genomic DNA from H69PR, H69, and H69AR cells. DNA (10 μg) was digested with Eco RI, analyzed by electrophoresis through a 0.7% agarose gel, and blotted onto a nitrocellulose membrane. The DNA was hybridized with a 1.8-kb Eco RI cDNA fragment of MRP that was labeled by random priming with [α-^{32}P]dCTP. The autoradiograph shown is a 6-hour exposure at −70°C. On the basis of the examination of several restriction digests and normalization of the amounts of DNA loaded, no differences in the copy number of the gene in H69 and H69PR cells were detected. Molecular size standards are shown at left. (**C**) Northern blot analysis of HeLa cell poly(A)$^+$ RNA with MRP cDNA. S3 is a drug-sensitive (S) HeLa cell line, and J2c is a drug-resistant (R) one (*28*). Poly(A)$^+$ RNA (2 μg) from each cell line was analyzed by electrophoresis, blotted, and probed with MRP cDNA as described in (A). The MRP and β-actin autoradiographs shown are 18-hour and 1-hour exposures, respectively, at −70°C.