Cell, Vol. 93, 1195–1205, June 26, 1998, Copyright ©1998 by Cell Press

Arabidopsis LEAFY COTYLEDON1 Is Sufficient to Induce Embryo Development in Vegetative Cells

Tamar Lotan,[1] Masa-aki Ohto,[1,5] Kelly Matsudaira Yee,[1] Marilyn A. L. West,[1,6] Russell Lo,[1,7] Raymond W. Kwong,[1] Kazutoshi Yamagishi,[1] Robert L. Fischer,[2] Robert B. Goldberg,[3] and John J. Harada[1,4]

[1]Section of Plant Biology
Division of Biological Sciences
University of California
Davis, California 95616

[2]Department of Plant and Microbial Biology
University of California
Berkeley, California 94720

[3]Department of Molecular, Cell, and Developmental Biology
University of California
Los Angeles, California 90024-1606

Summary

The *Arabidopsis LEAFY COTYLEDON1* (*LEC1*) gene is required for the specification of cotyledon identity and the completion of embryo maturation. We isolated the *LEC1* gene and showed that it functions at an early developmental stage to maintain embryonic cell fate. The *LEC1* gene encodes a transcription factor homolog, the CCAAT box–binding factor HAP3 subunit. *LEC1* RNA accumulates only during seed development in embryo cell types and in endosperm tissue. Ectopic postembryonic expression of the *LEC1* gene in vegetative cells induces the expression of embryo-specific genes and initiates formation of embryo-like structures. Our results suggest that LEC1 is an important regulator of embryo development that activates the transcription of genes required for both embryo morphogenesis and cellular differentiation.

Introduction

Higher plant embryogenesis is divided conceptually into two distinct phases: early morphogenetic processes that give rise to embryonic cell types, tissues, and organ systems, and late maturation events that allow the fully developed embryo to enter a desiccated and metabolically quiescent state (West and Harada, 1993; Goldberg et al., 1994). Upon reception of the appropriate signals, the dormant embryo germinates, and seedling development begins. Thus, seed maturation and metabolic quiescence interrupt the morphogenetic processes that occur during embryogenesis and seedling development. This unique form of development underlies, in part, a plant's ability to make seeds, a trait that has conferred significant selective advantages to higher plants (Steeves, 1983). Because lower plants do not make seeds and do not undergo embryo maturation, this bipartite mode of embryogenesis is thought to have resulted from the insertion of maturation events into the higher plant life cycle (reviewed by Walbot, 1978; Steeves and Sussex, 1989; Harada, 1997). Little is known at the mechanistic level about how distinct processes that occur during the morphogenesis and seed maturation phases are coordinated.

Genetic strategies employed to identify regulators of *Arabidopsis thaliana* embryo development have distinguished several gene classes that affect embryogenesis. One class of mutations, including *raspberry*, *tinman*, and *abnormal suspensor* (Schwartz et al., 1994; Yadegari et al., 1994; Devic et al., 1996; Tsugeki et al., 1996; T. L. et al, unpublished data), causes the arrest of embryo morphogenesis. Many of the corresponding proteins are involved in basic cellular functions and probably do not perform direct regulatory roles. A second class of genes predicted to play a role in embryonic pattern formation has been identified (Mayer et al., 1991). The expression patterns of two such genes, *EMB30* (*GNOM*) and *KNOLLE*, suggest that they do not function specifically during embryogenesis (Shevell et al., 1994; Lukowitz et al., 1996). Another class, including *SHOOTMERISTEMLESS* and *SCARECROW*, is active in the developing shoot or root apical meristems (Di Laurenzio et al., 1996; Long et al., 1996). Although these genes play key roles in meristem function, they are required for meristem formation during both embryogenesis and postembryonic development. *ABSCISIC ACID INSENSITIVE3* (*ABI3*), a fourth gene class, encodes an embryo-specific transcription factor that regulates genes expressed during seed maturation (Koornneef et al., 1984; Parcy et al., 1994). However, its function is limited to the late stages of embryogenesis. Although each of these gene classes is essential for embryo development, none appears to act specifically during embryogenesis to control both the morphogenesis and maturation phases.

The *LEAFY COTYLEDON1* (*LEC1*) gene, by contrast, controls many distinct aspects of embryogenesis (Meinke, 1992, 1994; West et al., 1994; Parcy et al., 1997). As summarized in Figure 1, the *lec1* mutation is pleiotropic, indicating several roles in late embryo development. *LEC1* is required for specific aspects of seed maturation. *lec1* mutant embryos are intolerant of desiccation and show defects in the expression of some, but not all, maturation-specific genes (Meinke, 1992; West et al., 1994; Parcy et al., 1997). *LEC1* is also involved in inhibiting premature germination. *lec1* mutant embryos exhibit morphological and molecular characteristics of both embryogenesis and postgerminative seedling development, showing that aspects of both programs can occur simultaneously (Meinke, 1992, 1994; West et al., 1994). *LEC1* plays a role in the specification of embryonic organ identity as well. Embryonic leaves or cotyledons of *lec1* mutants possess trichomes, epidermal hairs, which normally form only on leaves and stems

[4]To whom correspondence should be addressed.
[5]Present address: Department of Developmental Biology, National Institute for Basic Biology, Myodaijicho, Okazaki, 444 Japan.
[6]Present address: Department of Vegetable Crops, University of California, Davis, California 95616.
[7]Present address: Department of Genetics, University of Washington, Seattle, Washington 98195.

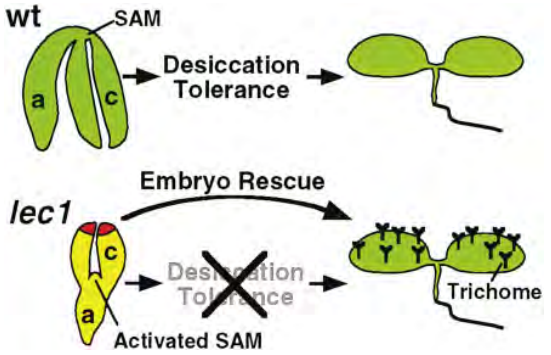

Figure 1. Pleiotropic Effects of the *lec1* Mutation on Embryo Development

Major differences between wild-type and *lec1* mutant embryos are as follows. Embryo shape: the axes of mutant embryos are short, and their cotyledons are round and do not curl. Anthocyanin generally accumulates at the tips of mutant cotyledons. Precocious germination: the shoot apical meristems of *lec1* embryos are activated in that they are domed and possess leaf primordia, unlike their wild-type counterparts that are flat and do not contain leaf primordia. Defects in seed maturation: *lec1* mutant embryos are intolerant of desiccation and normally die if dried on the plant. However, *lec1* embryos isolated before desiccation can be germinated to produce fertile homozygous mutant plants. The promoter of a 7S storage protein gene that is normally active during wild-type embryogenesis is not active in the *lec1* mutant. Incomplete specification of cotyledon identity: *lec1* seedlings possess trichomes on cotyledons. Trichomes are present on *Arabidopsis* leaves and stems but not on wild-type cotyledons. a, axis; c, cotyledon; SAM, shoot apical meristem.

in *Arabidopsis* (Meinke, 1992; West et al., 1994). The anatomy of *lec1* mutant cotyledons is intermediate between those of a wild-type cotyledon and a leaf (West et al., 1994). Finally, *LEC1* appears to act only during embryo development. Desiccation-intolerant *lec1* embryos can be rescued from plants before desiccation and germinated to produce homozygous mutant plants that are fertile and that do not display any obvious vegetative or floral mutant phenotypes (Meinke, 1992; West et al., 1994). Two other *LEC* class genes, *LEC2* and *FUSCA3* (*FUS3*), are thought to share similar or overlapping functions with *LEC1*, including the specification or cotyledon identity and the maintenance of maturation (Baumlein et al., 1994; Keith et al., 1994; Meinke et al., 1994). Although nothing has been reported about how *LEC* class genes act at the molecular level, their involvement in many diverse aspects of embryogenesis suggests that these genes serve as regulators of higher plant embryonic processes.

In this paper, we report the isolation of the *LEC1* gene and show that it encodes a homolog of a conserved eukaryotic transcription factor. Expression studies showed that the *LEC1* gene is active only within seeds during both early and late seed development. Ectopic expression of the *LEC1* gene induces embryonic programs and embryo development in vegetative cells. We suggest that LEC1 is an important transcriptional regulator required for both early and late embryogenesis that controls and coordinates higher plant embryo development.

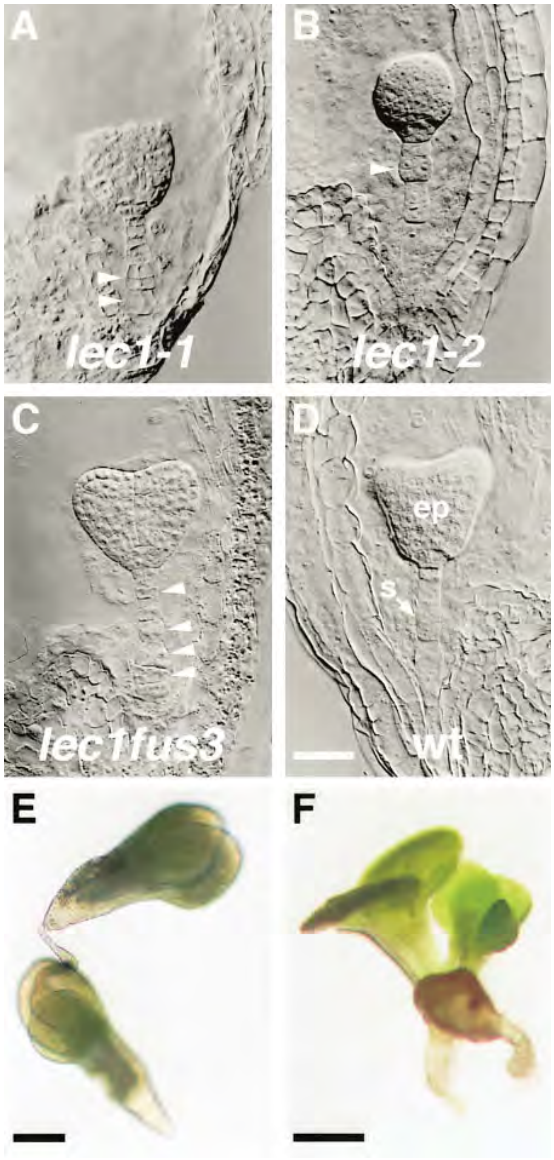

Figure 2. Embryogenic Potential of *lec1* Mutant Suspensor

(A)–(D) Seeds from the indicated plants were cleared and photographed using Nomarski optics. *lec1-1* (A); *lec1-2* (B); *lec1-2 fus3-3* (C); wild type (D). Arrowheads indicate sites of abnormal suspensor cell divisions. Bar, 25 μm. (E) A primary (top) and a secondary (bottom) embryo isolated from a *lec1-2 fus3-3* double mutant. Bar, 0.1 mm. (F) A germinated *lec1-2 fus3-3* seed containing twin seedlings. Bar, 0.5 mm. ep, embryo proper; s, suspensor.

Results

LEC1 Functions Early in Embryogenesis

The Lec1$^-$ phenotype indicates that the gene plays a significant role in controlling late embryo development (Figure 1). To determine whether the gene is also required for early embryonic events, we analyzed early-stage *lec1* mutant embryos and detected defects in suspensor morphology. The wild-type suspensor, shown in Figure 2D, is a transient embryonic structure consisting

Table 1. Suspensor Abnormalities in Mutant Embryos

	Embryonic Stages		
	Globular/Transition	Heart	Torpedo
Wild type	1/162 (0.01%)[a]	2/78 (2.6%)	0/200 (0%)
lec1-1	25/115 (22%)	87/128 (68%)	49/54 (91%)
lec1-2	15/164 (9.0%)	48/80 (60%)	29/32 (90%)
lec2	1/189 (0.01%)	4/47 (8.5%)	21/54 (39%)
fus3-3	9/97 (9.3%)	21/112 (19%)	10/89 (11%)
abi3-3	0/68 (0%)	0/62 (0%)	0/20 (0%)

[a]Number of embryos with abnormal suspensors/total embryos analyzed (percent embryos with abnormal suspensors).

of a single file of six to eight cells that are identical genotypically to embryo proper cells. By contrast, Figures 2A and 2B show that globular- and transition-stage embryos homozygous for either of the two *lec1* mutant alleles had abnormal suspensors. Cell walls parallel to the suspensor axis were observed, suggesting that aberrant cell divisions occurred in the mutant suspensors. As summarized in Table 1, abnormal suspensors rarely observed in wild-type embryos were detected initially in globular/transition-stage *lec1* embryos and were represented in approximately 90% of mutant torpedo-stage embryos.

We investigated a *lec1 fus3* double mutant (West et al., 1994) to learn whether other *LEC* class genes could enhance the effect of the *lec1* mutation on suspensor development. Figure 2C shows that suspensor abnormalities were observed in *lec1-2 fus3-3* double mutants at an early embryonic stage as with the single mutants. By contrast to wild-type suspensors that undergo a limited number of cell divisions, suspensor cells continued to proliferate in the double mutants. Subsequently, secondary embryos, shown in Figure 2E, formed from these abnormal suspensor cell masses in approximately 20% (118/598) of *lec1-2 fus3-3* seeds. Primary embryos were attached to secondary, suspensor-derived embryos either at or near the latter's shoot apices (Figure 2E) or at their root ends (data not shown). Both primary and secondary embryos were able to germinate, and viable seedings were produced (Figure 2F). Secondary embryo formation was also observed in *lec1-1 fus3-3* and *lec1-2 abi3-3* double mutants, but not in *lec1* single mutants or in *lec1-2 lec2* double mutants, and only rarely formed in *fus3-3* monogenic mutants (e.g., 2 of 298 seeds had secondary embryos). These results showed that polyembryony was not limited to a particular *lec1* or *fus3* allele. Control experiments showed that abnormal suspensors were also detected in early-stage *lec2-1* and *fus3-3* mutant embryos but not in *abi3-3* mutant embryos (Table 1). Together these results indicate that *LEC1* gene activity is required during early embryogenesis, in part, to suppress the embryogenic potential of the suspensor.

Insertion and Deletion Mutations Identify the *LEC1* Gene

lec1-1 and *lec1-2* mutant alleles were derived from a population of plants mutagenized insertionally with T-DNA (Feldmann and Marks, 1987; Meinke, 1992; West et al., 1994). The *lec1-1* mutation is not associated with

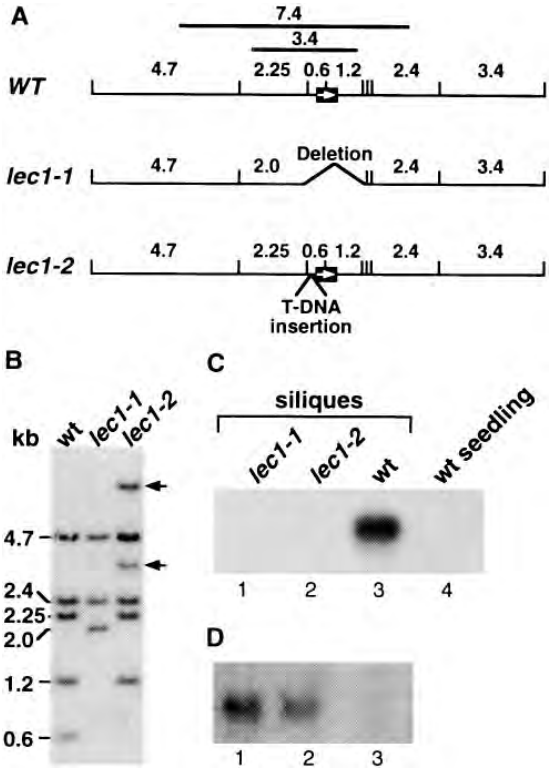

Figure 3. Structure and Expression of *LEC1* Gene Loci

(A) Diagrammatic representation of the *LEC1* locus in wild-type (WT), *lec1-1*, and *lec1-2* chromosomes. The box represents the LEC1 open reading frame and its transcriptional orientation. The positions of the deletion in *lec1-1* and of the T-DNA insertion in *lec1-2* are indicated. The bars represent the 3.4 kb BstYI restriction fragment used for the transgene complementation test and the 7.4 kb EcoRI restriction fragment used in (B). Vertical lines represent HindIII restriction sites.

(B) Gel blot of genomic DNA from wild-type (wt), *lec1-1*, and *lec1-2* digested with HindIII and hybridized with the wild-type 7.4 kb EcoRI fragment shown in (A). Arrows indicate restriction fragments in *lec1-2* that contain the T-DNA/plant DNA junctions.

(C) Gel blot containing 2 μg polyadenylated RNA from homozygous *lec1-1* siliques (lane 1), homozygous *lec1-2* siliques (lane 2), wild-type siliques (lane 3), and the leaves and stems of wild-type seedlings grown for 2 weeks (lane 4) were hybridized with the *LEC1* cDNA clone. The 0.85 kb *LEC1* RNA was detected only in wild-type siliques. Hybridization of this blot and the blot shown in (D) with a probe for a constitutively expressed ribosomal protein showed that equal amounts of RNA were present in each lane (data not shown).

(D) Gel blot analysis of 20 μg total RNA from wild-type siliques at three different stages hybridized with LEC1 cDNA clone. Lane 1, preglobular to heart stage; lane 2, heart to curled cotyledon stage; lane 3, maturing embryo stage.

T-DNA (Meinke, 1992; M. O. and J. J. H., unpublished data); however, we showed that a specific subset of the T-DNA fragments in *lec1-2* was within 1.5 cM of the *lec1* mutation. We identified genomic clones containing T-DNA sequences that cosegregated with the *lec1* mutation from a library of *lec1-2* DNA. The one clone that contained *Arabidopsis* DNA sequences identified restriction fragment length polymorphisms that distinguished wild-type, *lec1-1*, and *lec1-2* genomic DNAs. Figure 3B shows that plant DNA sequences flanking the

lec1-2 T-DNA identified 2.25 kb, 1.2 kb, and 0.6 kb HindIII fragments in wild-type DNA that were replaced with a 2.0 kb fragment in *lec1-1* DNA. The two *lec1-2* DNA fragments marked by the arrows in Figure 3B that were absent in wild-type DNA presumably represented T-DNA/plant DNA junction fragments.

Restriction mapping and nucleotide sequence analyses, summarized in Figure 3A, indicated that one T-DNA complex in *lec1-2* was inserted 115 bp upstream of a 626 bp open reading frame (ORF). Several lines of evidence suggested that this ORF represented the *LEC1* gene. First, as shown diagrammatically in Figure 3A, cloning and nucleotide sequencing studies showed that this ORF was part of a 2000 bp region deleted in *lec1-1* DNA. Second, this ORF was represented in embryo RNA. cDNA clones corresponding to the ORF were identified from a library of silique RNA that contained developing embryo mRNA. As shown in Figure 3C, this cDNA clone identified a single 0.85 kb polyadenylated RNA detected in wild-type silique RNA (lane 3) but not *lec1-1* (lane 1) or *lec1-2* (lane 2) silique RNAs. This result suggested that both mutations compromised the expression of this ORF. Third, RNA gel blot analysis using the 7.4 kb EcoRI restriction fragment shown in Figure 3A that spanned a region 4.4 kb upstream and 2.4 kp downstream of this ORF did not identify any other transcript in the silique RNA. Finally, nucleotide sequencing studies did not reveal any other extended ORFs within this 7.4 kb EcoRI restriction fragment.

To demonstrate directly that this ORF corresponded to the *LEC1* gene, we transferred the 3394 bp fragment that contained this ORF and its 5′ and 3′ flanking DNA sequences into homozygous *lec1-1* and *lec1-2* mutant plants (see Figure 3A). Plants transformed with this ORF produced viable seeds that survived desiccation, germinated, and produced fertile plants with a wild-type vegetative phenotype. Because *lec1* mutant embryos are normally desiccation intolerant, this result indicated that the ORF complemented the mutation. PCR amplification experiments and segregation analyses verified that all viable transgenic plants were homozygous for the *lec1* mutation and contained 1–3 copies of the transgene (data not shown). These data showed that the 3.4 kb fragment containing this ORF complemented *lec1* mutations. Taken together, these results indicate that the ORF represents the *LEC1* gene.

LEC1 Is a Homolog of CCAAT Box–Binding Factor

The predicted LEC1 polypeptide shared significant sequence similarity with the HAP3 subunit of the CCAAT box–binding factor (CBF; Figure 4). Based on amino acid sequence comparisons, the HAP3 subunit is divided into three domains: an amino-terminal A domain, a central B domain, and a carboxyl-terminal C domain, as shown diagrammatically in Figure 4A (Li et al., 1992). Figure 4B shows that LEC1 shared between 75% and 85% similarity and between 55% and 63% identity with the B domains of other HAP3 homologs. This degree of similarity was comparable to that obtained in sequence comparisons among HAP3 subunits from other organisms (Li et al., 1992). No significant sequence similarity was detected between the A and C domains of LEC1

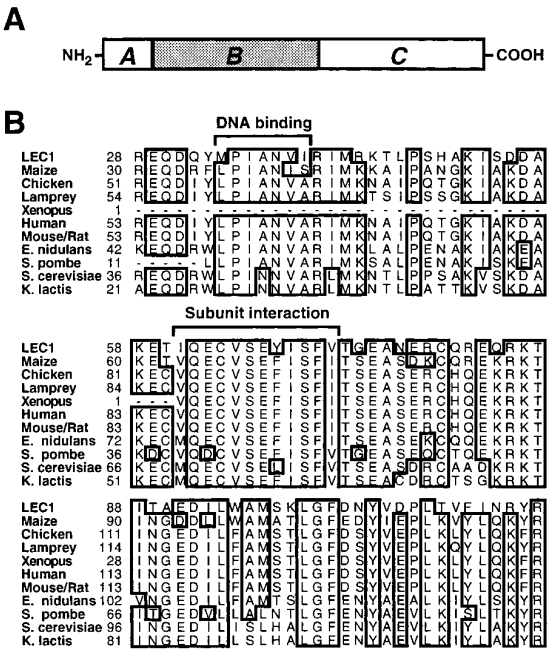

Figure 4. Amino Acid Sequence Similarity between LEC1 and Other CBF HAP3 Homologs

(A) Schematic representation of the three domains of the predicted LEC1 polypeptide.

(B) Comparison of the predicted amino acid sequence of the B domain encoded by *LEC1* with HAP3 homologs from maize, chicken, lamprey, *Xenopus laveis*, human, mouse (Li et al., 1992), rat (Vuorio et al., 1990), *Emericella nidulans* (Papagiannopoulos et al., 1996), *Schizosaccharomyces pombe* (Xing et al., 1993), *Saccharomyces cerevisiae* (Hahn et al., 1988), and *Kluyveromyces lactis* (Mulder et al., 1994). The DNA-binding region and the subunit interaction region are indicated. Numbers indicate amino acid positions of the B domains. The *Xenopus* sequence was derived from an incomplete cDNA clone.

and other HAP3 homologs, or with any other polypeptide sequence recorded in the databases. The high degree of sequence conservation in the B domain strongly suggests that LEC1 is part of an oligomeric CBF transcriptional activator.

LEC1 Is Expressed Specifically within Seeds during Early and Late Embryogenesis

We analyzed *LEC1* RNA levels to determine the expression pattern of the gene during development. Figure 3C shows that *LEC1* RNA was present in developing siliques of wild-type plants (lane 3) but was not detected in the leaves and stems of 2-week-old vegetative plants (lane 4). *LEC1* RNA was also not detected in gel blot hybridization experiments with polyadenylated RNAs from wild-type leaves, stems, roots, and flower buds (data not shown). Our previous observation that *lec1* mutant embryos rescued before desiccation occurred produce homozygous mutant plants with no obvious vegetative abnormalities is consistent with this apparent seed-specific expression pattern (Figure 1; West et al., 1994).

To define when the *LEC1* gene was active during embryogenesis, we measured *LEC1* RNA levels in siliques

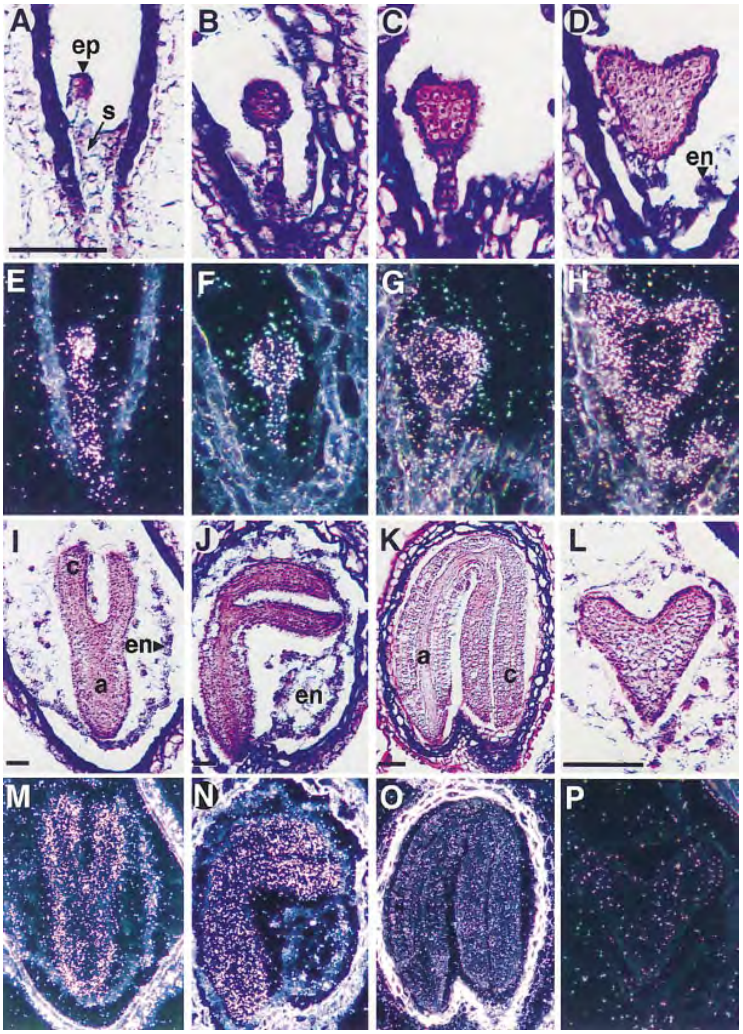

Figure 5. Distribution of *LEC1* mRNA in Developing Embryos

Wild-type and *lec1-1* mutant seed sections were hybridized with a *LEC1* antisense RNA probe and photographed following autoradiography. Sections (A)–(D) and (I)–(L) were stained with toluidine blue and photographed. Sections (E)–(H) and (M)–(P) were photographed with darkfield optics. As a negative control, the *LEC1* probe was hybridized to seed sections of *lec1-1* mutants. Hybridization with a *LEC1* sense RNA probe did not yield detectable signals (data not shown). Bars, 50 μm. a, embryonic axis; c, cotyledon; en, endosperm; ep, embryo proper; s, suspensor.

(A and E) Preglobular embryo.

(B and F) Globular-stage embryo.

(C and G) Transition-stage embryo.

(D and H) Heart-stage embryo.

(I and M) Torpedo/linear cotyledon–stage embryo.

(J and N) Bent cotyledon–stage embryo.

(K and O) Maturing embryo.

(L and P) *lec1-1* mutant heart–stage embryo.

at different stages of development. As shown in Figure 3D, *LEC1* RNA was present at higher levels in siliques containing early preglobular to heart-stage embryos (lane 1) and heart to curled cotyledon–stage embryos (lane 2) than in maturing embryos (lane 3). These results suggest that the *LEC1* gene is expressed at highest levels during early embryo development.

We hybridized a *LEC1* probe in situ with developing seed sections to determine the distribution of *LEC1* RNA within the seed. Figures 5A and 5E show that *LEC1* RNA accumulated in both the embryo proper and the suspensor of an early eight-celled proembryo. Other experiments showed that *LEC1* RNA was present shortly after fertilization within a two-celled proembryo and its suspensor (data not shown). *LEC1* RNA was present at a higher level in early-stage embryos at the proembryo stage, globular stage (Figures 5B and 5F), transition stage (Figures 5C and 5G), heart stage (Figures 5D and 5H), torpedo stage (Figures 5I and 5M), and curled cotyledon stage (Figures 5J and 5N) than in late maturing-stage embryos (Figures 5K and 5O). This result was consistent with the RNA gel blot studies (Figure 3D). Beginning with the globular and transition stages (Figures 5F and 5G), *LEC1* RNA became restricted to the embryo periphery, primarily within the outer protoderm and the ground tissue cell layers. By contrast, the extent of hybridization was much less in the procambial tissue at the center of the embryo proper (Figures 5G and 5H). The *LEC1* RNA accumulation pattern changed gradually during the progression from the linear to curled cotyledon stages when the RNA became distributed throughout the embryo (Figure 5N).

As shown in Figures 5H, 5M, and 5N, *LEC1* RNA also accumulated in the endosperm, a triploid nonembryonic seed tissue that originates from the fertilization of the central cell of a female gametophyte with a sperm nucleus. Figure 5 also shows that *LEC1* RNA was not detected in maternally derived silique and seed coat tissues, indicating that the *LEC1* RNA detected in silique RNA gel blots was present primarily within the seed (Figures 3C and 3D). Furthermore, Figures 5L and 5P show that the hybridization reactions were specific for *LEC1* RNA; no appreciable hybridization was observed within an early-stage seed of the *lec1-1* null mutant. The

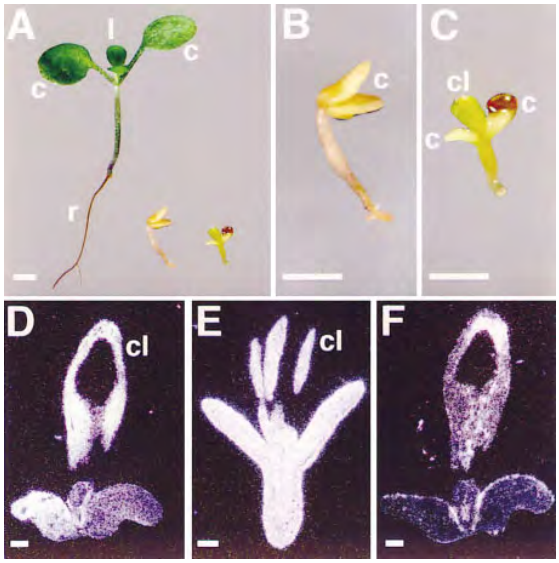

Figure 6. *35S/LEC1* Seedlings Have Embryonic Characteristics

(A) Comparison of a wild-type seedling grown for 2 weeks (left) with two *35S/LEC1* seedlings (right) that are shown at a higher magnification in (B) and (C).

(B) *35S/LEC1* seedling grown for 4 weeks that germinated but did not continue to develop.

(C) Two-week-old *35S/LEC1* seedling that produced a pair of cotyledon-like organs at the shoot apex.

(D–F) Darkfield micrographs of *35S/LEC1* seedling sections hybridized with probes for the following RNAs: (D) cruciferin A storage protein; (E) oleosin; (F) *LEC1*.

c, cotyledon; cl, cotyledon-like organ; l, leaf; r, root. Bars, 1 mm for (A)–(C) and 0.1 mm for (D)–(F).

pattern of *LEC1* gene expression and the *lec1* mutant phenotype suggest strongly that the *LEC1* gene functions specifically during embryogenesis, including the earliest embryonic period.

LEC1 Is Sufficient to Induce Embryonic Pathways

Because LEC1 appears to function as a specific regulator of embryo development, we wanted to know whether ectopic expression of the *LEC1* gene after embryogenesis affected vegetative development. We transferred a *LEC1* cDNA clone under the control of the cauliflower mosaic virus *35S* promoter into *lec1-1* null mutants. The *35S* promoter is active at a high level in most plant tissues (Odell et al., 1985).

We obtained viable, desiccated T1 seeds from *lec1-1* mutants transformed in planta with the 35S/*LEC1* construct. This result showed that the transgene complemented the mutation because *lec1* mutant seeds are intolerant of desiccation (Figure 1). However, viable seed production was a relatively rare event; T1 seeds germinated with an efficiency of only 0.006%, much less than the 1% efficiency typically obtained from in planta transformation experiments. Three-fourths of the seeds that germinated (33 of 43) produced T1 seedlings with abnormal terminal morphologies as shown in Figures 6B and 6C. These 35S/*LEC1* seedlings were smaller than wild-type seedlings (Figure 6A), and they possessed cotyledons that remained fleshy and failed to expand. Their roots often did not extend or extended only in sections and sometimes greened. As shown in Figure 6C, 35S/*LEC1* seedlings sometimes produced a single pair of cotyledon-like organs on the shoot apex at positions normally occupied by leaves. Unlike wild-type leaves, these organs did not expand and did not possess trichomes or mature stomatal structures (Figure 6C; data not shown). Morphologically, these organs closely resembled embryonic cotyledons.

Ten of the T1 35S/*LEC1* seedlings produced plants that grew vegetatively. One plant was male sterile and did not produce progeny. Seven other plants flowered and produced T2 progeny that all displayed the Lec1$^-$ phenotype though PCR amplification experiments confirmed the presence of the *35S/LEC1* transgene (data not shown). This result suggested that the 35S/*LEC1* gene was initially active in developing T1 seeds to complement the *lec1* mutation but that the gene became inactive after germination. Others have observed transgene silencing in plants (Matzke and Matzke, 1995; Stam et al., 1997). Two of the ten plants that grew vegetatively were exceptional in that they produced progeny with variable phenotypes. Only 25% of total desiccated seed from one plant, 20-3, was able to germinate, and all seedlings initially recapitulated the embryo-like seedling phenotypes shown in Figures 6B and 6C. All T2 progeny from the second, independently derived line, 21-4, also exhibited the embryo-like seedling phenotypes shown in Figures 6B and 6C. Thus, the ability of *35S/LEC1* plants to produce seedlings with an embryonic morphology was heritable to the T2 generation. T2 *35S/LEC1* plants that were fertile only produced progeny with a Lec1$^-$ phenotype.

Because the *35S/LEC1* seedlings had embryonic morphological characteristics, we asked whether they express genes normally active only in developing seeds. Figure 6D shows that cruciferin A storage protein RNA accumulated throughout 35S/*LEC1* T2 seedlings displaying embryonic characteristics, including the cotyledon-like organs at the position of leaves. Similar results were obtained in experiments with independently derived T1 embryo-like seedlings. We also showed that other embryo-specific RNAs encoding oleosin, an oil body protein (Figure 6E), and two 2S storage proteins (data not shown) accumulated similarly in these embryo-like seedlings. We confirmed that *LEC1* RNA accumulated in these 35S/*LEC1* seedlings (Figure 6F) but not in wild-type seedlings (data not shown; Figure 3C). Thus, *35S/LEC1* seedlings displaying an embryo-like phenotype accumulated embryo-specific RNAs. Together, these results suggest that ectopic *LEC1* gene expression induces embryonic programs in vegetative cells.

Of the T2 *35S/LEC1* seedlings that displayed embryonic characteristics (Figures 6B and 6C), most of the progeny from one line, 21-4, and approximately 10% from the second line, 20-3, continued to grow vegetatively, unlike the T1 seedlings that were arrested developmentally. These T2 plants displayed morphological abnormalities ranging from plants shown in Figure 7A with multiple embryonic cotyledon-like organs to plants with small, dark green abnormally shaped leaves that often produced callus-like cells. Immunochemical analysis showed that these plants accumulated cruciferin

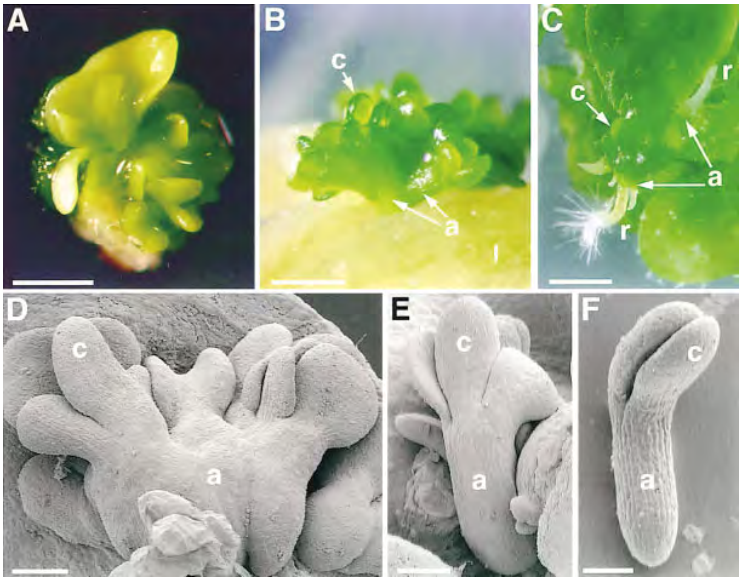

Figure 7. Embryo-like Structures on Transgenic Plants Ectopically Expressing the *LEC1* Gene

(A) *35S/LEC1* seedling that grew vegetatively and produced multiple cotyledon-like organs.
(B) Embryo-like structures on the leaf of a *35S/LEC1* plant that grew vegetatively.
(C) Axes of embryo-like structures that "germinated" to produce roots.
(D and E) SEM analysis of embryo-like structures. Structures resemble fused cotyledon-stage embryos with multiple cotyledons.
(F) SEM of wild-type cotyledon-stage embryo.
a, axis; c, cotyledon; l, leaf; r, root. Bars, 1 mm (A and C), 0.5 mm (B), 0.1 mm (D and E), and 0.05 mm (F).

storage protein (data not shown), suggesting that these vegetatively growing plants expressed embryonic programs.

A striking phenotype of the T2 progeny is shown in Figure 7B. We discovered embryo-like structures on the leaves of three progeny plants from the two independently derived *35S/LEC1* lines. As shown in Figure 7D, these structures resembled fused wild-type cotyledon-stage embryos (Figure 7F). Multiple embryonic cotyledon-like organs that lacked trichomes and mature stomata were attached to structures that resembled embryonic axes with elongated cells typical of wild-type embryos (Figure 7E; data not shown). Histological analyses suggested that, like wild-type embryos, these embryo-like structures possessed an outer protoderm layer, a central procambium layer, and ground tissue that consisted of several distinct files of cells (data not shown). Similar to embryos, the ground tissue cells of the ectopic embryos were densely cytoplasmic. Figure 7C shows roots that emerged from the axes tips, suggesting that these axes share similar functions with wild-type embryos. Finally, in situ hybridization experiments showed that embryo-specific RNAs encoding cruciferin A and 2S-1 storage proteins accumulated in these embryo-like structures but were not detected in the underlying leaf cells (data not shown). We conclude that post-embryonic expression of the *LEC1* gene is sufficient to induce embryo formation in vegetative tissues of these two lines.

Discussion

LEC1 Is a Transcriptional Activator Homolog

We have shown that the LEC1 polypeptide is homologous to the HAP3 subunit of the CBF class of eukaryotic transcriptional activators that includes NF-Y, CP1, and HAP2/3/4/5 (Johnson and McKnight, 1989). The sequence similarity between LEC1 and other HAP3 subunits is restricted to the B domain, consistent with the finding that this domain is conserved evolutionarily (Li et al., 1992). Furthermore, amino acid residues of yeast and mammalian HAP3 subunits required for DNA binding and for interactions with other CBF subunits are conserved in LEC1 (Figure 4; Xing et al., 1993; Sinha et al., 1996). Experiments demonstrating that yeast and mammalian CBF subunits can be combined to form DNA-binding complexes indicate that this amino acid sequence similarity underlies functional conservation (Chodosh et al., 1988; Sinha et al., 1995).

CBFs are heteroligomeric transcription factors, but it is not known whether the plant CBF is organized as its yeast counterpart into four nonhomologous subunits, HAP2, 3, 4, and 5, or is similar to the trimeric mammalian CBF (Maity et al., 1992; McNabb et al., 1994). Oilseed rape, maize, and *Arabidopsis* DNA sequences encoding HAP2, HAP3, and HAP5 homologs have been identified, although the functional roles of the plant proteins have not been established (Li et al., 1992; Newman et al., 1994; Albani and Robert, 1995). Several distinct *Arabidopsis* DNA sequences have been identified that correspond to each of these subunits, including the LEC1/HAP3 subunit, implicating the existence of gene families.

Because LEC1 is a component of a plant CBF, we predict that it regulates embryonic processes by activating the transcription of specific genes. Mammalian CBFs are thought to serve a general role in transcription by optimizing promoter efficiency through its binding with CCAAT DNA sequences that are found 50–100 bp upstream of many mammalian genes (Myers et al., 1986). However, some mammalian CBFs have been shown to increase the transcriptional activities of specific genes by their association with other transcription factors (Wright et al., 1994; Ericsson et al., 1996). The CBF containing LEC1 is unlikely to serve a general role in transcription for several reasons. First, CBFs in other organisms regulate specific gene sets. For example, yeast CBFs specifically activate genes encoding mitochondrial proteins involved in respiration (Guarente et al.,

1984; Keng and Guarente, 1987; Trueblood et al., 1988; Schneider and Guarente, 1991). Second, CCAAT boxes are not typically found upstream of most plant genes near position −80, and mutation of the CCAAT sequence within the 35S promoter does not affect promoter activity or footprint formation (Benfey and Chua, 1990). Third, *lec1* null mutations, although pleiotropic, do not abrogate the transcription of many genes, including those encoding cruciferin A, oleosin, and late embryogenesis abundant proteins (West et al., 1994). Thus, the Lec1$^-$ phenotype most likely results from abnormal transcription of specific genes regulated by the CBF containing LEC1. Finally, expression of the 35S/*LEC1* gene in postembryonic plants induces embryonic processes, indicating that specific gene sets required for embryo development are activated by LEC1 (Figures 6 and 7). We conclude that LEC1 is a specific transcriptional regulator of genes required for normal *Arabidopsis* embryo development.

LEC1 Is a Central Regulator of Embryogenesis

A key to define the precise role of LEC1 in embryo development is to understand whether LEC1 functions throughout embryogenesis or specifically during either the morphogenesis phase or the maturation phase. We and others speculated previously that LEC1 might function solely during morphogenesis as a homeotic regulator of cotyledon identity (Meinke, 1992; West et al., 1994). Cotyledons and leaves are homologous organs. Incomplete specification of organ identity resulting from the *lec1* mutation could cause cotyledons to acquire leaf characteristics and defects in seed maturation (Figure 1). However, *LEC1* RNA is distributed throughout the embryo and in the endosperm but not exclusively in developing cotyledons (Figure 5). These results suggest that *LEC1* does not act solely in the specification of cotyledon identity. An alternative hypothesis is that LEC1 might act exclusively to regulate the maturation phase of embryogenesis (West et al., 1994; Parcy et al., 1997). Because germination is actively suppressed during embryo development (Harada, 1997), defects in maturation processes are expected to cause premature activation of postgerminative development. Thus, the phenotype of *lec1* mutants (Figure 1) could result from the heterochronic effects of the mutation. Our finding that *LEC1* is expressed early in embryogenesis and is required for early embryo development (Figures 2 and 3) indicates that LEC1 functions before the onset of maturation and, therefore, cannot be involved only in regulating this late embryonic phase. The simplest interpretation is that LEC1 plays a more central role in embryo development.

The ability of *LEC1* to induce embryonic programs in vegetative cells establishes the gene as a critical regulator of embryogenesis. The seed maturation phase of embryogenesis is induced, at least in part, in seedlings expressing the gene as indicated by the activation of cruciferin A and 2S storage protein genes and the oleosin gene (Figure 6). The failure of *35S/LEC1* seedlings with embryonic characteristics (Figure 6) to continue vegetative development is also consistent with induction of maturation because morphogenesis is normally arrested during the seed maturation phase (Harada, 1997). Because the *LEC1* gene is not normally expressed postembryonically, this result suggests that LEC1 may activate genes that suppress vegetative development. Our previous finding that postgerminative development is activated prematurely in *lec1* mutant embryos is consistent with this intepretation (West et al., 1994). Alternatively, LEC1 may interfere with interactions between transcription factors in seedlings, creating a dominant-negative mutation that disrupts vegetative development. The effect of *LEC1* gene expression on postembryonic development may explain the low germination frequency of *35S/LEC1* T1 seeds and the rarity with which we recovered vegetative plants with embryonic structures. The *35S/LEC1* transgene must be active in the developing *lec1* mutant seeds to permit the completion of embryo development, yet the activity of the *LEC1* gene is antagonistic to vegetative development. Thus, continued overexpression of the *35S/LEC1* gene following germination is likely to inhibit vegetative development, and silencing of the transgene probably produced plants with the Lec1$^-$ phenotype. Because transgene activity is highly variable in independent transformants (Matzke and Matzke, 1995; Stam et al., 1997), only those rare transgenic plants producing LEC1 protein at a critical level may be competent to produce vegetative plants with embryonic structures. Together, these results indicate that LEC1 is sufficient to induce many aspects of the maturation phase of embryogenesis in vegetative cells.

The formation of embryo-like structures on the leaves of *35S/LEC1* plants (Figure 7) strongly suggests that LEC1 is sufficient to induce the morphogenesis phase of embryo development, although it remains to be determined how closely ectopic embryo formation follows zygotic embryogenesis. Additional evidence that ectopic *LEC1* gene expression induces embryonic structures comes from preliminary experiments showing that embryonic cotyledon-like structures form on seedlings ectopically expressing the *LEC1* gene from a different promoter (R. W. K. and J. J. H., unpublished results). Our findings that the *lec1* mutation causes defects in suspensor morphology in early-stage embryos (Figure 2) and that the *LEC1* gene is expressed at the earliest stages of embryo development (Figure 5) provide independent evidence of *LEC1* function early in embryogenesis. This conclusion is also consistent with genetic analyses of *lec1 lec2* double mutants. Although *lec1* and *lec2* mutants at their terminal stages resemble late-stage embryos, the double mutant arrests with the morphology of an early torpedo-stage embryo, suggesting a role for both genes in early embryogenesis (data not shown; Meinke et al., 1994).

The ability of LEC1 to induce both the morphogenesis and maturation phases of embryogenesis and to suppress vegetative development suggests its fundamental role in regulating different aspects of embryogenesis. As discussed above, other genes shown to be required for embryo development do not function seed-specifically throughout embryogenesis. *LEC1* is the only gene shown to be sufficient to induce embryo formation in vegetatively growing plants. For example, *ABI3* has been implicated to play a critical role in controlling the late seed maturation phase of embryogenesis. Transgenic plants containing a *35S/ABI3* gene express a subset of genes normally active during seed maturation

when challenged with the hormone ABA (Parcy et al., 1994). Ectopic *ABI3* gene expression does not cause visible defects in vegetative or reproductive development in these transgenic plants, suggesting that ABI3 is not sufficient to induce the maturation phase. This result suggests that, unlike *LEC1*, the role of *ABI3* is more limited to regulating gene sets expressed during seed maturation. Double mutant analyses indicating that *LEC1* and *ABI3* do not appear to act in series in the same genetic pathway are consistent with this interpretation (data not shown; Meinke et al., 1994). We note that it has recently been reported that embryonic programs are induced in vegetative root cells of the *pickle* mutant (Ogas et al., 1997).

Considering that LEC1 can induce embryo development in vegetative cells, why is the Lec1$^-$ phenotype not more severe? Although *lec1* mutant embryos are intolerant of desiccation, they continue to undergo many aspects of morphogenesis and seed maturation (see Figure 1). A clue comes from analyses of cruciferin A, oleosin, and 2S storage protein gene expression. These genes are expressed in *35S/LEC1* seedlings, yet they are also active in *lec1* mutant embryos (Figure 6; West et al., 1994). Thus, LEC1 is sufficient but not necessary for their expression, implicating genetic redundancy. That is, another gene, possibly the other *LEC* genes *LEC2* and/or *FUS3*, may partially fulfill LEC1 function during embryogenesis.

In conclusion, we have shown that LEC1 is a transcription factor homolog that is required early and late in embryo development and that it is sufficient to induce embryonic programs in vegetative cells. Together, our results suggest that LEC1 is a major embryonic regulator that mediates the switch between embryo and vegetative development. LEC1's role in inducing and maintaining embryogenesis while suppressing vegetative development is likely to be critical for the establishment of the seed habit of higher plants. Given this central role, the *LEC1* gene and its protein will be important tools for the dissection of higher plant embryogenesis. In particular, one key will be to identify the downstream genes regulated by LEC1 and the protein(s) with which it interacts in establishing the regulatory circuits controlling embryonic development.

Experimental Procedures

Plant Material

lec1-1, *lec1-2*, and *lec2-1* mutants were derived from a population of *Arabidopsis thaliana* ecotype Wassilewskija (Ws-O) lines mutagenized with T-DNA insertions (Feldmann and Marks, 1987; Meinke, 1992, 1994; West et al., 1994). *abi3-3* and *fus3-3* mutants and *lec2-1* were provided by Peter McCourt (University of Toronto; Nambara et al., 1992; Keith et al., 1994) and by David Meinke (Oklahoma State University). Plants were grown as described previously (West et al., 1994). Digenic mutants were constructed and their genotypes were verified through backcrosses with each parental line as described previously (West et al., 1994).

Isolation and Sequence Analysis of Genomic and cDNA Clones

Genomic DNA libraries from homozygous *lec1-1* and *lec1-2* mutants were constructed in the λGEM 11 vector (Promega). A wild-type Ws-O genomic library was provided by Ken Feldmann (University of Arizona). Two cDNA libraries were prepared according to manufacturers' specifications in the λZAPII bacteriophage vector (Stratagene) from wild-type siliques containing globular- to heart-stage embryos and heart- to young torpedo-stage embryos.

Clones were isolated from the *lec1-2* genomic library using probes for the right and left T-DNA borders. A 7.1 kb XhoI fragment containing the plant DNA/T-DNA junction was isolated and cloned into the Bluescript-KS plasmid (Stratagene) to create pML7. A 4.2 kb *EcoR*I fragment containing plant DNA from pML7 was used to isolate genomic clones from a wild-type Ws-0 library. A 7.4 kb *EcoR*I fragment present in several overlapping clones was inserted into the Bluescript-KS plasmid and used to identify corresponding clones from a *lec1-1* genomic library and from the wild-type silique cDNA libraries. Eighteen *LEC1* cDNA clones were isolated and entirely or partially sequenced; all sequences were identical to corresponding regions of the *LEC1* gene. Additional details of the cloning experiments are available upon request. Nucleotide sequencing was done using the automated dideoxy chain termination method on an ABI Prism 377 DNA Sequencer. Database searches were performed at the National Center for Biotechnology Information by using the BLAST network service. Alignment of protein sequences was done using PILEUP program (Genetics Computer Group, Madison, WI).

Production of Transgenic *lec1* Mutants

A 3.4 kb BstYI fragment containing the wild-type *LEC1* gene was inserted into the plant transformation vector, pBIB-Hyg (Becker, 1990). A full-length *LEC1* cDNA was fused in the proper transcriptional orientation with the 35S promoter and the octopine synthase terminator into the plasmid, pART7 (Gleave, 1992). The entire fusion gene was transferred into the plant transformation vector BJ49. Constructs were transferred into homozygous *lec1-1* and/or *lec1-2* mutants using the in planta transformation procedure with *Agrobacterium tumefaciens* strain GV3101 (Bechtold et al., 1993). Genotypes of the complemented plants were verified in DNA amplification experiments. T2 plants containing the *35S/LEC1* transgene were either germinated from dry seeds or rescued from siliques and grown on basal media (Olsen et al., 1993).

DNA and RNA Hybridization Analyses

Nucleic acid isolation and gel blot hybridization experiments were done as described previously (West et al., 1994). In situ hybridization experiments were performed as described previously (Dietrich et al., 1989).

Scanning Electron Microscopy

SEM analysis was performed as described previously (Yadegari et al., 1994).

Acknowledgments

We thank Jay Danao, Minsung Kim, and Sara Wortley for their help with experiments, Bart Janssen for vectors, K.C. McFarland of the NSF-Plant Cell Biology Training Facility for help in generating figures, and Neelima Sinha for comments about the manuscript. T. L. was supported by a postdoctoral fellowship from the Human Frontiers Science Program, M. O. was supported by postdoctoral fellowships for research abroad from the Japanese Society for the Promotion of Science and from Kenzo Nakamura, National Institute of Basic Biology, and K. Y. was supported in part by a postdoctoral fellowship from the Japanese Society for the Promotion of Science. This work was supported by a grant from the Department of Energy.

Received December 9, 1997; revised May 7, 1998.

References

Albani, D., and Robert, L.S. (1995). Cloning and characterization of a *Brassica napus* gene encoding a homologue of the B subunit of a heteromeric CCAAT-binding factor. Gene *167*, 209–213.

Baumlein, H., Misera, S., Luerben, H., Kolle, K., Horstmann, C., Wobus, U., and Muller, A.J. (1994). The *FUS3* gene of *Arabidopsis thaliana* is a regulator of gene expression during late embryogenesis. Plant J. *6*, 379–387.

Bechtold, N., Ellis, J., and Pelletier, G. (1993). *In planta Agrobacterium* mediated gene transfer by infiltration of adult *Arabidopsis thaliana* plants. C.R. Acad. Sci. Paris *316*, 1194–1199.

Becker, D. (1990). Binary vectors which allow the exchange of plant selectable markers and reporter genes. Nucleic Acids Res. *18*, 203.

Benfey, P.N., and Chua, N.-H. (1990). The cauliflower mosaic virus 35S promoter: combinatorial regulation of transcription in plants. Science *250*, 959–966.

Chodosh, L.A., Olesen, J., Hahn, S., Baldwin, A.S., Guarente, L., and Sharp, P.A. (1988). A yeast and a human CCAAT-binding protein have heterologous subunits that are functionally interchangeable. Cell *53*, 25–36.

Devic, M., Albert, S., and Delseny, M. (1996). Induction and expression of seed-specific promoters in Arabidopsis embryo-defective mutants. Plant J. *9*, 205–215.

Dietrich, R.A., Maslyar, D.J., Heupel, R.C., and Harada, J.J. (1989). Spatial patterns of gene expression in *Brassica napus* seedlings: identification of a cortex-specific gene and localization of messenger RNA encoding isocitrate lyase and a polypeptide homologous to proteinases. Plant Cell *1*, 73–80.

Di Laurenzio, L., Wysocka-Diller, J., Malamy, J.E., Pysh, L., Helariutta, Y., Freshour, G., Hahn, M.G., Feldmann, K.A., and Benfey, P.N. (1996). The SCARECROW gene regulates an asymmetric cell division that is essential for generating the radial organization of the *Arabidopsis* root. Cell *86*, 423–433.

Ericsson, J., Jackson, S.M., and Edwards, P.A. (1996). Synergistic binding of sterol regulatory element-binding protein and NF-Y to the farnesyl diphosphate synthase promoter is critical for sterol-regulated expression of the gene. J. Biol. Chem. *271*, 24359–24364.

Feldmann, K.A., and Marks, M.D. (1987). Agrobacterium-mediated transformation of germinating seeds of *Arabidopsis thaliana*: a non-tissue culture approach. Mol. Gen. Genet. *208*, 1–9.

Gleave, A.P. (1992). A versatile binary vector system with a T-DNA organisational structure conducive to efficient integration of cloned DNA into the plant genome. Plant Mol. Biol. *20*, 1203–1207.

Goldberg, R.B., De Paiva, G., and Yadegari, R. (1994). Plant embryogenesis: zygote to seed. Science *266*, 605–614.

Guarente, L., Lalonde, B., Gifford, P., and Alani, E. (1984). Distinctly regulated tandem upstream activation sites mediate catabolite repression of the CYC1 genes of *S. cerevisiae*. Cell *32*, 317–321.

Hahn, S., Pinkham, J., Wei, R., Miller, R., and Guarente, L. (1988). The HAP3 regulatory locus of Saccharomyces cerevisiae encodes divergent overlapping transcripts. Mol. Cell. Biol. *8*, 655–663.

Harada, J.J. (1997). Seed maturation and control of germination. In Advances in Cellular and Molecular Biology of Plants, Volume *4*, Cellular and Molecular Biology of Seed Development, B.A. Larkins and I.K. Vasi, eds. (Dordrecht: Kluwer Academic Publishers), pp. 545–592.

Johnson, P.F., and McKnight, S L. (1989). Eukaryotic transcriptional regulatory proteins. Annu. Rev. Biochem. *58*, 799–840.

Keith, K., Kraml, M., Dengler, N.G., and McCourt, P. (1994). *fusca3*: a heterochronic mutation affecting late embryo development in Arabidopsis. Plant Cell *6*, 589–600.

Keng, T., and Guarente, L. (1987). Constitutive expression of the yeast *HEM1* gene is actually a composite of activation and repression. Proc. Natl. Acad. Sci. USA *84*, 9113–9117.

Koornneef, M., Reuling, G., and Karssen, C.M. (1984). The isolation and characterization of abscisic acid-insensitive mutants of *Arabidopsis thaliana*. Physiol. Plant *61*, 377–383.

Li, X.-Y., Mantvani, R., Hooft Van Huijsduijnen, R., Andre, I., Benoist, C., and Mathis, D. (1992). Evolutionary variation of the CCAAT-binding transcription factor NF-Y. Nucleic Acids Res. *20*, 1087–1091.

Long, J.A., Moan, E.I., Medford, J.I., and Barton, M.K. (1996). A member of the KNOTTED class of homeodomain proteins encoded by the STM gene of Arabidopsis. Nature *379*, 66–69.

Lukowitz, W., Mayer, U., and Juergens, G. (1996). Cytokinesis in the *Arabidopsis* embryo involves the syntaxin-related KNOLLE gene product. Cell *84*, 61–71.

Maity, S.N., Sinha, S., Ruteshouser, E.C., and De Crombrugghe, B. (1992). Three different polypeptides are necessary for DNA binding of the mammalian heteromeric CCAAT binding factor. J. Biol. Chem. *267*, 16574–16580.

Matzke, M.A., and Matzke, A.J.M. (1995). How and why do plants inactivate homologous (trans)genes? Plant Physiol. *107*, 679–685.

Mayer, U., Torres Ruiz, R.A.T., Berleth, T., Misera, S., and Jurgens, G. (1991). Mutations affecting body organization in the *Arabidopsis* embryo. Nature *353*, 402–407.

McNabb, D.S., Xing, Y., and Guarente, L. (1994). Cloning of yeast HAP5: a novel subunit of a heterotrimeric complex required for CCAAT binding. Genes Dev. *9*, 47–58.

Meinke, D.W. (1992). A homoeotic mutant of *Arabidopsis thaliana* with leafy cotyledons. Science *258*, 1647–1650.

Meinke, D.W., Franzmann, L.H., Nickle, T.C., and Yeung, E.C. (1994). *leafy cotyledon* mutants of Arabidopsis. Plant Cell *6*, 1049–1064.

Mulder, W., Scholten, I.H., de Boer, R.W., and Grivell, L.A. (1994). Sequence of the HAP3 transcription factor of *Kluyveromyces lactis* predicts the presence of a novel 4-cysteine zinc-finger motif. Mol. Gen. Genet. *245*, 96–106.

Myers, R.M., Tilly, K., and Maniatis, T. (1986). Fine structure genetic analysis of a beta-globin promoter. Science *232*, 613–618.

Nambara, E., Naito, S., and McCourt, P. (1992). A mutant of Arabidopsis which is defective in seed development and storage protein accumulation is a new *abi3* allele. Plant J. *2*, 435–441.

Newman, T., De Bruijn, F.J., Green, P., Keegstra, K., Kende, H., McIntosh, L., Ohlrogge, J., Raikhel, N., Somerville, S., Thomashow, M., et al. (1994). Genes galore: a summary of methods for accessing results from large-scale partial sequencing of anonymous Arabidopsis cDNA clones. Plant Physiol. *106*, 1241–1255.

Odell, J.T., Nagy, F., and Chua, N.-H. (1985). Identification of DNA sequences required for activity of cauliflower mosaic virus 35S promoter. Nature *313*, 810–812.

Ogas, J., Cheng, J.-C., Sung, Z.R., and Somerville, C. (1997). Cellular differentiation regulated by gibberellin in the *Arabidopsis thaliana pickle* mutant. Science *277*, 91–94.

Olsen, L.J., Ettinger, W.F., Damsz, B., Matsudaira, K.L., Webb, M.A., and Harada, J.J. (1993). Targeting of glyoxysomal proteins to peroxisomes in leaves and roots of a higher plant. Plant Cell *5*, 941–952.

Papagiannopoulos, P., Andrianopoulos, A., Sharp, J.A., Davis, M.A., and Hynes, M.J. (1996). The hapC gene of Aspergillus nidulans is involved in the expression of CCAAT-containing promoters. Mol. Gen. Genet. *251*, 412–421.

Parcy, F., Valon, C., Raynal, M., Gaubier-Comella, P., Delseny, M., and Giraudat, J. (1994). Regulation of gene expression programs during Arabidopsis seed development: roles of the *ABI3* locus and of endogenous abscisic acid. Plant Cell *6*, 1567–1582.

Parcy, F., Valon, C., Kohara, A., Misera, S., and Giraudat, J. (1997). The *ABSCISIC ACID-INSENSITIVE3*, *FUSCA3*, and *LEAFY COTYLEDON1* loci act in concert to control multiple aspects of Arabidopsis seed development. Plant Cell *9*, 1265–1277.

Schneider, J.C., and Guarente, L. (1991). Regulation of the yeast *CYT1* gene encoding cytochrome c_1 by HAP1 and HAP2/3/4. Mol. Cell Biol. *11*, 4934–4942.

Schwartz, B.W., Yeung, E.C., and Meinke, D.W. (1994). Disruption of morphogenesis and transformation of the suspensor in abnormal suspensor mutants of *Arabidopsis*. Development *120*, 3235–3245.

Shevell, D.E., Leu, W.-M., Gillmor, C.S., Xia, G., Feldmann, K.A., and Chua, N.-H. (1994). *EMB30* is essential for normal cell division, cell expansion, and cell adhesion in arabidopsis and encodes a protein that has similarity to Sec7. Cell *77*, 1051–1062.

Sinha, S., Maity, S.N., Lu, J., and de Crombrugghe, B. (1995). Recombinant rat CBF-C, the third subunit of CBF/NFY, allows formation of a protein-DNA complex with CBF-A and CBF-B and with yeast HAP2 and HAP3. Proc. Natl. Acad. Sci. USA *92*, 1624–1628.

Sinha, S., Kim, I.S., Sohn, K.-Y., de Crombrugghe, B., and Maity, S.N. (1996). Three classes of mutations in the A subunit of the CCAAT-binding factor CBF delineate functional domains involved in the three-step assembly of the CBF-DNA complex. Mol. Cell. Biol. *16*, 328–337.

Stam, M., Mol, J.N.M., and Kooster, J.M. (1997). The silence of genes in transgenic plants. Ann. Bot. *79*, 3–12.

Steeves, T.A. (1983). The evolution and biological significance of seeds. Can. J. Bot. *61*, 3550–3560.

Steeves, T.A., and Sussex, I.M. (1989). Patterns in Plant Development, Second Edition (Cambridge: Cambridge University Press).

Trueblood, C.E., Wright, R.M., and Poyton, R.O. (1988). Differential regulation of the two genes encoding *Saccharomyces cervisiae* cytochrome oxidase subunit V by heme and *HAP2* and *REO1* genes. Mol. Cell Biol. *8*, 4537–4740.

Tsugeki, R., Kochieva, E.Z., and Fedoroff, N.V. (1996). A transposon insertion in the Arabidopsis SSR16 gene causes an embryo-defective lethal mutation. Plant J. *10*, 479–489.

Vuorio, T., Maity, S.N., and de Crombrugghe, B. (1990). Purification and molecular cloning of the "A" chain of a rat. J. Biol. Chem. *265*, 22480–22486.

Walbot, V. (1978). Control mechanisms for plant embryogeny. In Dormancy and Developmental Arrest, M.E. Clutter, ed. (New York: Academic Press, Inc.), pp. 113–166.

West, M.A., and Harada, J.J. (1993). Embryogenesis in higher plants: an overview. Plant Cell *5*, 1361–1369.

West, M.A.L., Matsudaira Yee, K.L., Danao, J., Zimmerman, J.L., Fischer, R.L., Goldberg, R.B., and Harada, J.J. (1994). *LEAFY COTYLEDON1* is an essential regulator of late embryogenesis and cotyledon identity in Arabidopsis. Plant Cell *6*, 1731–1745.

Wright, K.L., Vilen, B.J., Itoh-Lindstrom, Y., Moore, T.L., Li, G., Criscitiello, M., Cogswell, P., Clarke, J.B., and Ting, J.P.-Y. (1994). CCAAT box binding protein NF-Y facilitates in vivo recruitment of upstream DNA binding transcription factors. EMBO J. *13*, 4042–4053.

Xing, Y., Fikes, J.D., and Guarente, L. (1993). Mutations in yeast HAP2/HAP3 define a hybrid CCAAT box binding domain. EMBO J. *12*, 4647–4655.

Yadegari, R., De Paiva, G.R., Laux, T., Koltunow, A.M., Apuya, N., Zimmerman, J.L., Fischer, R.L., Harada, J.J., and Goldberg, R.B. (1994). Cell differentiation and morphogenesis are uncoupled in Arabidopsis *raspberry* embryos. Plant Cell *6*, 1713–1729.

GenBank Accession Number

The accession number for the sequence reported in this paper is AF036684.