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AGL15, a MADS Domain Protein Expressed in Developing Embryos

Gregory R. Heck, Sharyn E. Perry, Karl W. Nichols, and Donna E. Fernandez[1]
Department of Botany, University of Wisconsin, 430 Lincoln Drive, Madison, Wisconsin 53706-1381

To extend our knowledge of genes expressed during early embryogenesis, the differential display technique was used to identify and isolate mRNA sequences that accumulate preferentially in young *Brassica napus* embryos. One of these genes encodes a new member of the MADS domain family of regulatory proteins; it has been designated *AGL15* (for AGAMOUS-like). *AGL15* shows a novel pattern of expression that is distinct from those of previously characterized family members. RNA gel blot analyses and in situ hybridization techniques were used to demonstrate that *AGL15* mRNA accumulated primarily in the embryo and was present in all embryonic tissues, beginning at least as early as late globular stage in *B. napus*. Genomic and cDNA clones corresponding to two *AGL15* genes from *B. napus* and the homologous single-copy gene from Arabidopsis, which is located on chromosome 5, were isolated and analyzed. Antibodies prepared against overexpressed Brassica AGL15 lacking the conserved MADS domain were used to probe immunoblots, and AGL15-related proteins were found in embryos of a variety of angiosperms, including plants as distantly related as maize. Based on these data, we suggest that AGL15 is likely to be an important component of the regulatory circuitry directing seed-specific processes in the developing embryo.

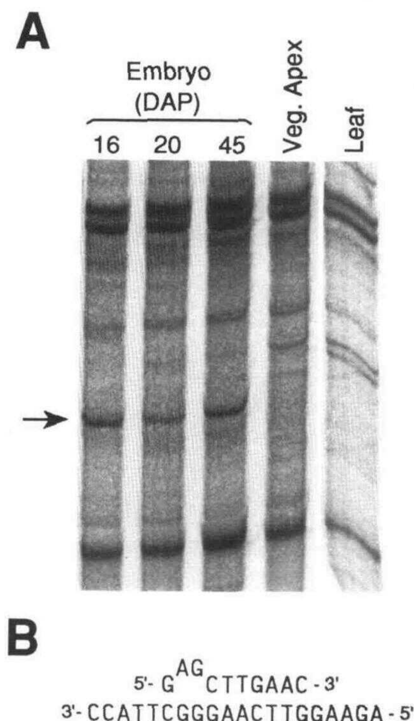
INTRODUCTION

Higher plants have a unique developmental phase that we recognize as the seed. The evolution of the seed habit represented a significant departure from the life cycle of bryophytes and seedless vascular plants, in which development from zygote to mature sporophyte is essentially continuous. To establish the young sporophyte, suspend its growth, and then release the quiescent state in a controlled manner at some later point require the correct spatial and temporal regulation of several different developmental programs during embryo development and seed maturation. These programs include morphogenesis, which establishes embryo form; overlapping programs related to the "seed habit" and cytodifferentiation, which drive processes such as the stage-specific accumulation of storage reserves and development of desiccation tolerance; and postgermination programs, which must be actively repressed until appropriate germination conditions occur. Variations in the specific nature, time of onset, and duration of these programs generate a diverse array of embryogenic patterns in flowering plants.

The number of genes whose expression must be modulated to achieve successful embryogeny and seed maturation is large. In recent years, much work has focused on genetic and molecular analyses (reviewed in West and Harada, 1993; Goldberg et al., 1994) of members of two particular families: the mustard family, Brassicaceae (e.g., Arabidopsis, Brassica, and Capsella), and members of the grass family, Poaceae (e.g., maize and other cereals). These families represent the dicot and monocot lineages, respectively. Analyses of Arabidopsis (Franzmann et al., 1995) or maize (Sheridan and Clark, 1993) mutants have identified hundreds of genetic loci that, when disrupted, yield arrested or developmentally abnormal embryos in stages ranging from fertilization to seed maturity. Many of the mutations affect the earliest stages of embryogenesis before bilateral symmetry appears. As expected, some of these early defects are due to disruptions in basic cellular metabolism. For example, Arabidopsis embryos homozygous for the *bio1* mutation can complete embryo development only if they are supplied with exogenous biotin (Schneider et al., 1989). Analyses of other mutants have provided insights into the establishment of embryo polarity and pattern (Mayer et al., 1991), the interaction between programs of morphogenesis and cytodifferentiation (Yadegari et al., 1994), and the relationship between seed development and postgermination phases of the life cycle (Baumlein et al., 1994; Keith et al., 1994; Meinke et al., 1994; West et al., 1994; Nambara et al., 1995).

Sequence information is available for very few of the loci shown by genetic means to be important during early embryogenesis; therefore, little is known about gene products that perform essential functions during this period. Molecular markers useful in studying effects of mutant loci are also scarce, particularly markers for early developmental stages before the onset of cytodifferentiation (Lindsey and Topping, 1993). To identify genes expressed in young embryos, we took advantage of the relatively large size of *Brassica napus* (oilseed rape)

[1] To whom correspondence should be addressed.



B

5'- G^{AG}CTTGAAC - 3'
3'- CCATTCGGGAACTTGGAAGA - 5'

Figure 1. Differential Display of *AGL15*.

(A) PCR products visualized on a portion of a differential display autoradiogram. *AGL15* appears as an amplified band (arrow) in samples derived from transition-stage (16 DAP), heart-stage (20 DAP), or maturation-stage (45 DAP) *B. napus* embryos but not in samples derived from vegetative apices (Veg. Apex) or mature leaf.
(B) Proposed binding site for the 5′ arbitrary primer (5′-GAGCTTGAAC-3′) on the *B. napus AGL15-1* cDNA sequence, which is located 485 bases upstream of the polyadenylation site. Superscripts indicate sites of base pair mismatches.

embryos, the high degree of sequence identity between Brassica and Arabidopsis genes, and polymerase chain reaction (PCR) techniques. In the course of this work, we identified and isolated *AGL15* (for AGAMOUS-like), which encodes a putaive regulatory protein that may play a significant role in the embryo development of dicots, such as Arabidopsis, and possibly all angiosperms.

AGL15 is a new member of the MADS box family of transcriptional regulators. This regulatory family is defined by a conserved DNA binding domain, and representatives have been found in all major eukaryotic lineages, including yeast, arthropods, vertebrates, and plants. Seventeen different Arabidopsis MADS box genes have been isolated and characterized to date (Ma et al., 1991; Rounsley et al., 1995). Most of these genes are preferentially expressed in developing flowers. The floral genes encode important regulators of meristem identity, such as *APETELA1* (*AP1*; Mandel et al., 1992b) and *CAULIFLOWER* (*CAL*; Kempin et al., 1995), and factors involved in the specification of organ identity within floral whorls, such as *AGAMOUS* (*AG*; Yanofsky et al., 1990), *APETELA3* (*AP3*; Jack et al., 1992), and *PISTILLATA* (*PI*; Goto and Meyerowitz, 1994). A new class of MADS box genes, which is expressed primarily in developmental contexts other than the flower, was recently identified by Rounsley et al. (1995). *AGL15*, which was isolated in our laboratory on the basis of its preferential expression in developing embryos, is a member of this new class. The temporal and spatial pattern of mRNA accumulation and the conservation of structural elements in the *AGL15* gene product during evolution indicate that *AGL15* is likely to be an important component of the regulatory circuitry directing seed-specific processes in young plant embryos.

RESULTS

Isolation of a Low-Abundance mRNA Preferentially Expressed in Developing Embryos Using Differential Display

To identify genes that are preferentially expressed in young embryos, the differential display method of Liang and Pardee (1992) was used to compare mRNA sequences present in developing embryos of *B. napus* at the transition and heart stages with mRNA sequences present in older embryos, the postgermination shoot apex, and mature leaves. The differential display profiles of amplified sequences derived from young embryos (transition, heart, and torpedo stages at 16 to 26 days after pollination [DAP]) were most similar overall to the profiles of amplified sequences derived from shoot apex mRNA (data not shown). The young embryo and shoot apex profiles were distinct from those derived from embryo stages or organs in which differentiation was more advanced, such as in maturation-stage embryos (45 DAP) and fully expanded leaves, which in turn were also different from each other (data not shown). One particular amplification product that was derived from the priming oligonucleotides 5′-T_{12}CG-3′ and 5′-GAGCTTGAAC-3′ appeared to be present only in samples from developing embryos, as shown in Figure 1A. This amplification product (~500 bp) was cloned and used to screen a cDNA library prepared from transition-stage (16- to 19-DAP) *B. napus* embryos. The sequence appeared at a frequency of ~0.02% in the unamplified library, suggesting that the mRNA is in a low-abundance class. Ten positive clones were isolated.

Sequence Analysis and Identification of *AGL15*, a New Member of the MADS Domain Gene Family

Sequences from several of the isolated cDNA clones were analyzed. The priming site for the arbitrary 5′ oligonucleotide used in the differential display was found 485 bp from the polyadenylation site. As indicated in Figure 1B, this decamer acted as a hexamer or heptamer in the initial amplification; this is consistent with the observations of Liang and Pardee (1992).

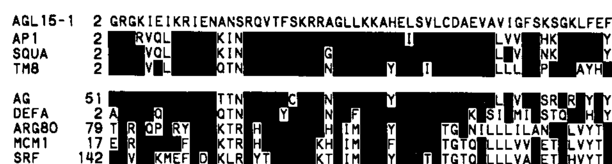


Figure 2. Comparison of Deduced Amino Acid Sequences of MADS Domains of *B. napus* AGL15-1 and Selected MADS Gene Family Members.

The MADS domain encoded by *B. napus AGL15-1* is presented on the top line. Identical residues in the MADS domains of other proteins are indicated by the black boxes. Numbering at left indicates the positions of the first residues shown within their respective proteins. The AGL15-1 MADS domain most closely matches that of AP1 from Arabidopsis (Mandel et al., 1992b), SQUA from Antirrhinum (Huijser et al., 1992), and TM8 from tomato (Pnueli et al., 1991). A comparison with the sequences used to define the MADS domain originally is also shown: AG from Arabidopsis (Yanofsky et al., 1990), DEFICIENS A (DEFA) from Antirrhinum (Sommer et al., 1990), arginine80 (ARG80) from yeast (Dubois et al., 1987), minichromosome maintenance1 (MCM1) from yeast (Passmore et al., 1988), and serum response factor (SRF) from humans (Norman et al., 1988).

The full-length open reading frame of the completely spliced message is 795 bp and encodes a predicted 30-kD protein of 264 amino acids. Protein data base comparisons indicate strong homologies with a family of both known and putative transcriptional regulators found in a wide range of eukaryotes, collectively known as MADS domain proteins (Schwarz-Sommer et al., 1990). Residues 2 to 57 of the deduced amino acid sequence of the *B. napus* protein shared more than 70% identity with the MADS domains from a variety of plant genes. The most closely matching amino acid sequences are aligned in Figure 2 and include SQUAMOSA (SQUA; 80%) from Antirrhinum (Huijser et al., 1992), AP1 (80%) from Arabidopsis (Mandel et al., 1992b), and TM8 (for tomato MADS box 8; 73%) from tomato (Pnueli et al., 1991).

In addition to the highly conserved MADS domain, the predicted amino acid sequence shows homology with plant family members in a second region, the K domain (Ma et al., 1991; Pnueli et al., 1991). K domains exhibit less conservation of primary sequence but share a putative amphipathic α-helical structure that may be involved in facilitating interactions between proteins (Ma et al., 1991). Residues 92 to 157 of the Brassica sequence could also form amphipathic α-helices according to secondary structure predictions and have significant, albeit lower, levels of identity with the K domain of SQUA (43%) and AP1 (38%). As in other MADS domain proteins, the C terminus (residues 158 to 264) of the predicted Brassica protein is divergent and shows no significant homologies with SQUA, AP1, other MADS gene family members, or with any other sequences found in the data base. Thus, on the basis of sequence analysis, we concluded that we had isolated a new member of the MADS gene family. In accordance with the numbering scheme of Rounsley et al. (1995), this gene was subsequently designated *AGL15*. The gene corresponding to the first cDNA species that we characterized was designated *B. napus AGL15-1*.

Temporal and Spatial Patterns of Expression

To verify the observed differential display pattern, the accumulation of mRNA corresponding to *AGL15* was examined by RNA gel blot analysis. Total RNA was isolated from a variety of developmental stages and organs of *B. napus*, and blots were probed with a region of the cDNA downstream of the conserved MADS domain. As shown in Figure 3A, the probe hybridized to a single size class of transcripts of ~1000 bases, which accumulated predominantly in developing embryos. *AGL15* mRNA could be detected in all isolated embryo samples, starting with the earliest age examined, heart stage, and continuing through late maturation. Because it is well represented in the transition-stage library, *AGL15* mRNA must accumulate at least as early as late globular stage in Brassica. Accumulation of *AGL15* mRNA peaked at approximately torpedo stage and then fell gradually through the remainder of embryo maturation (complete maturity is at ~60 DAP). After prolonged exposure (20 days) of the RNA gel blot shown in Figure 3A (2-day exposure), *AGL15* transcripts could also be detected in other tissues. Levels of accumulation in apices of the inflorescence (including buds ≤1 mm), young floral buds equivalent to stage 9 in Arabidopsis floral development, young seedlings, and vegetative apices were at least 10-fold lower than those in embryos (data not shown). Transcripts were present at very low levels in roots but were undetectable in mature leaf samples.

The expression pattern of *AGL15* in *B. napus* did not match that of any of the MADS box genes previously identified in the closely related plant Arabidopsis (Yanofsky et al., 1990; Ma et al., 1991; Jack et al., 1992; Mandel et al., 1992b; Goto and Meyerowitz, 1994; Kempin et al., 1995) but showed at least a partial overlap with the expression pattern of *AGL2*. Using in situ hybridization, Flanagan and Ma (1994) showed that *AGL2* transcripts accumulate in the embryo, developing floral organs, and ovules. To determine whether *AGL2* and *AGL15* transcripts accumulate at the same time, poly(A)$^+$ RNA was isolated from various *B. napus* tissues and hybridized either with an *AGL15* probe generated from the *B. napus AGL15-1* cDNA sequence or an *AGL2* probe generated from the Arabidopsis cDNA sequence. As shown in Figure 3B, the expression pattern of *AGL15* is significantly different from that of *AGL2*. The *AGL2* transcripts accumulated to relatively high levels in samples dominated (on the basis of tissue mass) by nonembryonic tissues, such as the mature carpel and 10-DAP whole seed. *AGL2* mRNAs also accumulated in the inflorescence, at levels approximately twofold lower than those in the seed, but could not be detected in poly(A)$^+$ RNA isolated from torpedo-stage embryos or in total RNA isolated from heart-stage embryos (data not shown). In contrast, *AGL15* transcripts were readily detectable in torpedo-stage embryos, could not be detected in seed coat or carpel tissue, and accumulated in the

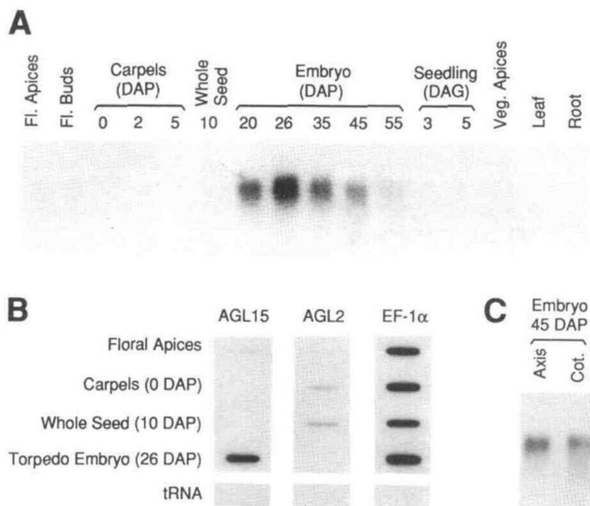


Figure 3. *AGL15* mRNA Accumulation Patterns in *B. napus.*

(A) RNA gel blot analysis. Total RNA from a variety of organs and developmental stages was hybridized with the *B. napus AGL15-1* cDNA sequence downstream of the MADS domain (probe 2). Fl. Apices, inflorescence meristems and buds up to 1 mm; Fl. Buds, 1- to 2-mm floral buds (equivalent to stage 9 in Arabidopsis; Smyth et al., 1990; Scott et al., 1991); Whole Seed, collected at 10 DAP; Veg. Apices, vegetative meristems and leaf primordia up to 2 mm; DAG, days after germination.

(B) Comparison of *AGL2* and *AGL15* mRNA accumulation patterns. Slot blots were loaded with equal amounts of poly(A)$^+$ RNA or tRNA and hybridized with *AGL15*-specific (probe 2) or *AGL2*-specific probes (30-hr exposures). The *AGL15* blot was stripped and rehybridized with an EF-1α–specific probe (3-hr exposure) to test for equal loading of RNA.

(C) RNA gel blot analysis of total RNA isolated from either the axes or cotyledons of maturation-stage embryos (45 DAP) and hybridized with an *AGL15*-specific probe (probe 2). Cot., cotyledon.

inflorescence at levels ~15-fold lower than levels in the embryo. We conclude that only *AGL15* shows strong preferential expression in embryonic tissues in *B. napus.*

In developing flowers, mRNAs encoding AG, AP1, and other MADS domain proteins accumulate preferentially in particular organs. To ascertain whether *AGL15* mRNAs are similarly restricted in distribution within developing embryos, a combination of RNA gel blot analysis and in situ hybridization was used. Total RNA was isolated separately from cotyledons and axes of maturation-stage embryos (45 DAP), and RNA gel blots were probed with *AGL15*-specific sequences. Figure 3C shows that *AGL15* transcripts were present in both organs and accumulated to approximately equal levels. When embryo sections were hybridized with ^{35}S-labeled antisense RNA probes, *AGL15* mRNAs could be detected throughout the torpedo-stage embryo (26 DAP) and, as shown in Figure 4A, accumulated in all tissues and cell types. Additional embryo sections hybridized with RNA probes corresponding to the sense strand showed only background levels of labeling, as seen in Figure 4B. To determine whether *AGL15* mRNAs accumulated to equally high levels but in a more restricted set of cells in the developing flower, sections of developing inflorescence were hybridized with the antisense probe. After long exposures, some *AGL15* mRNA accumulation could be detected in the cortex of the inflorescence axis, but no signal above background was detected in the apical meristem of the inflorescence or in developing flowers (data not shown).

Genomic Structure and Complexity of *AGL15* in *B. napus*

AGL15 expression in the *B. napus* embryo is the product of a complex amphidiploid genome. Further analysis of the *AGL15* cDNAs isolated from the unamplified transition-stage library revealed the presence of a second species of cDNA. The gene corresponding to this cDNA was designated *B. napus AGL15-2.* The deduced amino acid sequence of *B. napus* AGL15-2 is 83% identical overall to that of *B. napus* AGL15-1. Restriction fragments corresponding to the two distinct *AGL15* genes (*AGL15-1* and *AGL15-2*) in *B. napus* were identified by probing genomic DNA gel blots with the *AGL15-1* cDNA sequence downstream of the MADS domain. In the blot shown in Figure 5A, at least two strongly hybridizing fragments and several other restriction fragments that hybridized less strongly can be seen. Based on preliminary characterization of clones isolated from a cultivar Bridger genomic library, at least some of the minor

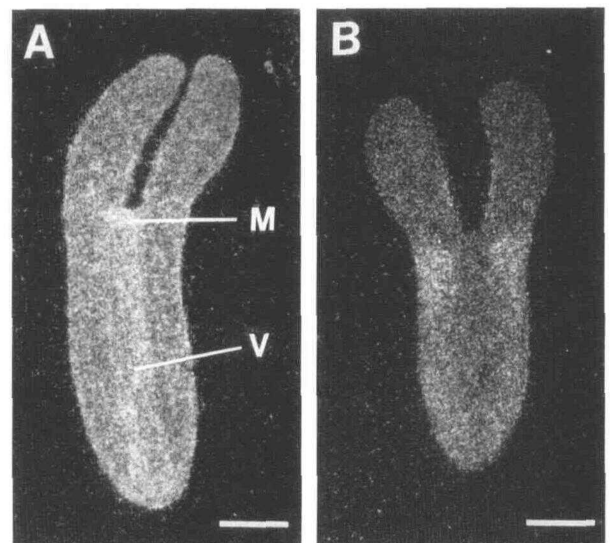


Figure 4. Localization of *AGL15* mRNA in Torpedo-Stage *B. napus* Embryos at 26 DAP.

(A) Dark-field image of a medial longitudinal section probed with the *AGL15-1* antisense RNA probe (probe 3). M, shoot apical meristem; V, provascular tissue.

(B) Dark-field image of a longitudinal section probed with the *AGL15-1* sense RNA probe (probe 4) showing background levels of labeling. Bars = 150 μm.

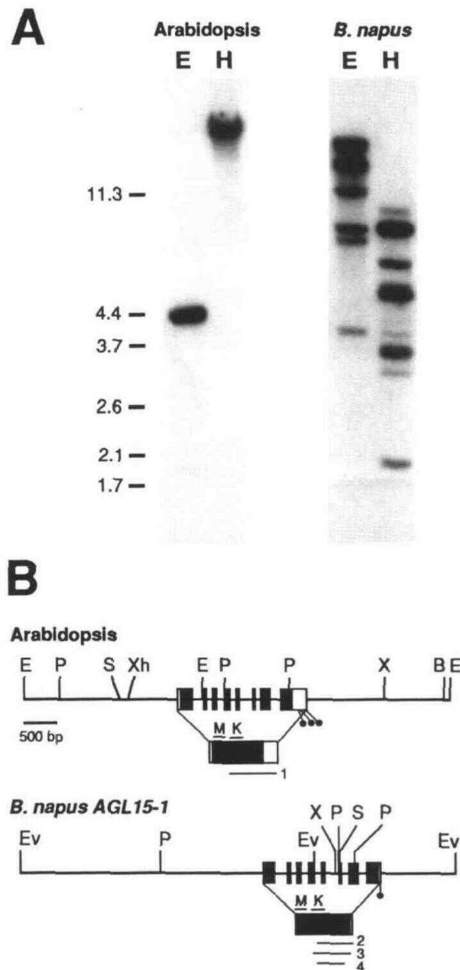


Figure 5. Structure and Copy Number of Arabidopsis and *B. napus* *AGL15* Genes.

(A) Gel blots of Arabidopsis and *B. napus* genomic DNA digested with restriction enzymes and hybridized with the corresponding (Arabidopsis or *B. napus*) *AGL15* cDNA sequence downstream of the MADS domain (probes 1 and 2). The positions of the length markers are indicated at left in kilobases. E, EcoRI digest; H, HindIII digest.

(B) Schematic diagrams of the Arabidopsis *AGL15* and *B. napus AGL15-1* loci. Exons are represented by boxes, with black shading indicating translated sequence. Polyadenylation sites are indicated by dots. The corresponding cDNAs are shown below each gene with the MADS domain (M) and K box domain (K) indicated with bars. Sequences used as probes for various analyses are indicated as well. Probe 1, Arabidopsis cDNA downstream of the MADS domain; probe 2, *B. napus* cDNA downstream of the MADS domain; probe 3, antisense probe used for in situ analysis; probe 4, sense probe used for in situ analysis. Restriction sites are designated as follows: B, BamHI; E, EcoRI; Ev, EcoRV; P, PstI; S, SnaBI; X, XbaI; Xh, XhoI.

fragments appeared to be pseudogene derivatives of the *AGL15* genes. The genomic clone corresponding to *B. napus AGL15-1* is diagrammed in Figure 5B. In terms of the number and placement of the introns, the Brassica *AGL15* genes are most similar structurally to *AP1* and *SQUA*. Because these same genes also show the most identity in deduced amino acid sequence in both the MADS and K domains, the *AP1* MADS gene subfamily may be considered the closest relatives to *AGL15*.

Identification of an Arabidopsis *AGL15* Homolog

To facilitate the genetic analysis of *AGL15* function, a homolog of the *B. napus AGL15* genes was isolated from Arabidopsis. A developing silique cDNA library was probed using the *AGL15-1* cDNA sequence downstream of the MADS box (Figure 5B, probe 2) under stringent conditions, and several full-length cDNA clones were obtained. The Arabidopsis cDNA sequence (Figure 5B, probe 1) was then used to probe an Arabidopsis genomic library and blots of genomic DNA. As shown in Figure 5A, genomic DNA blot analysis and low-stringency hybridizations (data not shown) confirmed that this gene represents a single locus in Arabidopsis. The genomic clone corresponding to the Arabidopsis *AGL15* locus is diagrammed in Figure 5B. The Arabidopsis *AGL15* locus is very similar structurally to the Brassica *AGL15* loci. Reverse transcription–PCR using isolated Arabidopsis embryos (G.R. Heck and D.E. Fernandez, unpublished results) and in situ hybridization (Rounsley et al., 1995) indicated that, as in *B. napus*, transcripts from this gene are present in developing embryos.

The predicted amino acid sequences of Arabidopsis AGL15, *B. napus* AGL15-1, and *B. napus* AGL15-2 can be aligned with only small deletions/insertions and amino acid changes for most of the length of the proteins, as shown in Figure 6A. Arabidopsis AGL15 and *B. napus* AGL15-1 share 95% amino acid identity in the MADS domain, 71% in the K domain, and 75% at the C-terminal end. As seen in Figure 6B, at least part of the divergence in the C terminus of the gene products can be attributed to a frameshift in the Arabidopsis *AGL15* open reading frame. Relative to the *B. napus* sequences, which remain aligned throughout this region, the Arabidopsis sequence contains four additional nucleotides within the codon for Arg-248. The resulting frameshift adds 16 additional amino acids to the C terminus of Arabidopsis AGL15. In the absence of the inserted nucleotides, the Arabidopsis sequence would translate into a C terminus that is identical to the C terminus of *B. napus* AGL15-1. Beyond the stop codon used in *B. napus*, the nucleotide sequences of the Arabidopsis gene and corresponding *B. napus* untranslated region diverge.

Chromosomal Location of *AGL15* in Arabidopsis

The location of the *AGL15* locus was mapped relative to previously identified markers in the Arabidopsis genome. Segregation of nucleotide polymorphisms within the *AGL15* sequence was examined in pooled progeny of F_2 families derived from a cross between the Columbia and Landsberg *erecta* ecotypes. As a result, *AGL15* could be positioned on the top of chromosome 5 near the restriction fragment length polymorphism

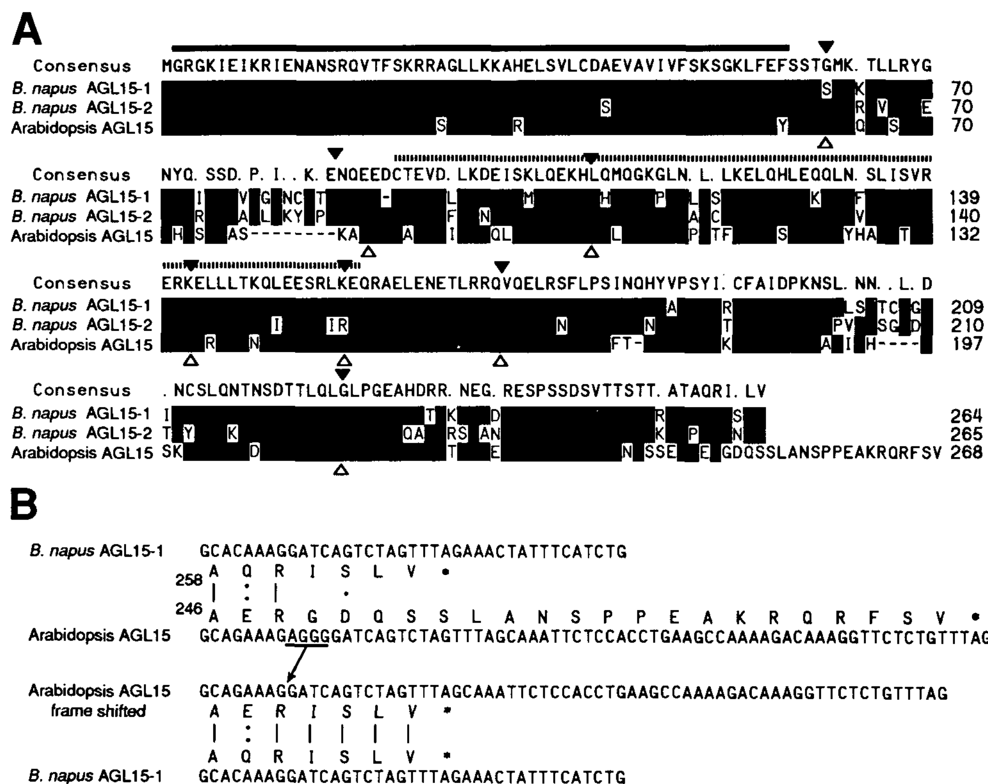


Figure 6. Alignment of Deduced Amino Acid Sequences of AGL15 from *B. napus* and Arabidopsis.

(A) Comparison of amino acid sequences encoded by two *B. napus AGL15* genes (*B. napus AGL15-1* and *AGL15-2*) and the single Arabidopsis *AGL15* gene. A consensus sequence, indicating identity between at least two sequences, is presented on the top line. Amino acids identical to the consensus are indicated by the black boxes. Dashes indicate gaps inserted to maximize alignment. The MADS domain (solid bar), K box domain (hatched bar), and positions of introns (*B. napus*, solid triangles from above; Arabidopsis, open triangles from below) are indicated. GenBank accession numbers for corresponding nucleotide sequences are as follows: *B. napus AGL15-1* (U22665) and *AGL15-2* (U22681) and Arabidopsis *AGL15* (U22528).

(B) Effect of a frameshift on the C terminus of the Arabidopsis AGL15 protein. The top pair of sequences compares the seven C-terminal amino acids of *B. napus* AGL15-1 and the 23 C-terminal residues of Arabidopsis AGL15 as deduced from the cDNA sequence. Identical amino acids are indicated by vertical bars and decreasing degrees of similarity by two dots and one dot. Asterisks indicate stop codons. To obtain the lower pair of sequences, the extra four bases that appear within the Arabidopsis codon for Arg-248 (relative to the *B. napus* sequences) were removed. The C terminus of the gene product derived from this hypothetical sequence would be identical to the C terminus of the *B. napus* AGL15-1 protein.

(RFLP) marker pCITd37, located at position 21.4 on an integrated genetic/RFLP map (Hauge et al., 1993). Mapping at greater resolution was achieved by hybridization to ordered yeast artificial chromosomes (YACs) spanning this region. In Figure 7, *AGL15* and the order of representative, positively hybridizing YACs are shown. *AGL15* hybridized to the YACs EW6C7 and EW17B2 but not to yUP10A10. Because EW6C7 hybridized to *AGL15* but not the nearby RFLP markers pCITd37 and chs (for chalcone synthase), *AGL15* can be placed unambiguously less than 200 kb distal to chs, the *transparent testa 4* (*tt4*) locus. Visible markers flanking *AGL15* thus include *lutescens* (*lu*) and *tt4*.

A defect in embryo development might be expected from a mutation disrupting the *AGL15* locus. Although large collections of embryo-defective (*emb*) mutants have been isolated, only ~20% of the total estimated number of single-gene mutations that give rise to embryo-defective phenotypes have been mapped. The closest of the *EMB* loci in this region is *EMB262* (Franzmann et al., 1995). Mutations in the *EMB262* locus yield embryos arrested in the linear-curled cotyledon stage. Because *emb262* is putatively tagged by a T-DNA insertion (Castle et al., 1993), DNA was isolated from wild-type and *emb262* heterozygous plants and probed with *AGL15* and several kilobases of the surrounding genomic sequence. No large polymorphisms were detected in heterozygous plants. Therefore, unless a second mutation that was undetectable by genomic DNA hybridization is closely linked to the T-DNA insertion, it is unlikely that the *EMB262* locus encodes the *AGL15* gene product.

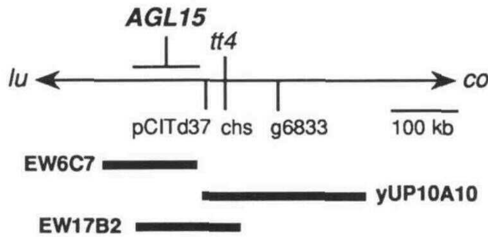


Figure 7. Chromosomal Location of *AGL15* in Arabidopsis.

A portion of the top of chromosome 5 surrounding the *AGL15* locus is shown with relative positions of visible markers (*lu*, *tt4*, and *co* at map positions 17.6, 20.1, and 20.3, respectively; Hauge et al., 1993), RFLP markers (pCITd37, chs, and g6833; Putterill et al., 1993), and ordered YAC clones (EW6C7, yUP10A10, and EW17B2; Putterill et al., 1993).

Conservation of AGL15 Structural Elements within the Angiosperms

If the *AGL15* gene product plays an important role in embryo development, we might expect to find a related protein(s) performing homologous functions in embryos of many different groups of flowering plants. To test whether structural elements of the protein (apart from the MADS domain) are conserved, polyclonal antibodies were prepared against the K domain and C-terminal regions of Brassica AGL15-1, affinity purified, and used on protein gel blots. Antibodies prepared in this way are highly specific for AGL15 (either Brassica or Arabidopsis): they do not recognize overexpressed forms of other MADS domain proteins, such as AGL2, or any soluble proteins in tissues expected to lack AGL15, such as Brassica mature leaf (S.E. Perry and D.E. Fernandez, unpublished results). As shown in Figure 8, these antibodies recognized a low-abundance protein (< 0.01% total soluble fraction) of the expected relative molecular mass (30 kD) within soluble extracts of young *B. napus* embryos. The antibodies were then used in a survey of embryos or whole seeds in plants belonging to different angiosperm lineages. As shown in Figure 8, strongly immunoreactive proteins were seen in papaya, a representative of a family within the same order as Brassica; pepper, a representative of the more distantly related dicot family Solanaceae; and maize, a representative of the monocot lineage. All of the dicot representatives had an immunoreactive 30-kD species. Papaya embryos contained additional immunoreactive species, which could have resulted from AGL15 degradation and/or cross-reaction with a novel protein. Maize embryos also contained a single immunoreactive species, which was ~6 kD larger than that in dicots. Therefore, structural elements outside of the DNA binding domain appear to be strongly conserved. This suggests that other regions of the protein may be important for function and that there may have been selection pressure to maintain AGL15 structure and function as different groups of flowering plants evolved.

DISCUSSION

We isolated and characterized a gene, *AGL15*, that encodes a putative transcription factor expressed in young plant embryos. Very few genes that are expressed before heart stage, which marks the onset of differentiation processes in the embryo, have been identified previously (Lindsey and Topping, 1993). Using differential display, we isolated a collection of sequences expressed during the transition from globular to heart stage in *B. napus*. Of the sequences we analyzed, only *AGL15* had the pattern of expression we might expect for a factor involved in embryo specification. Using AGL15-specific antibodies, we established that the AGL15 protein is present in whole *B. napus* seed at least as early as 6 DAP (early globular stage; S.E. Perry and D.E. Fernandez, unpublished results). Arabidopsis *AGL15* was independently isolated by Rounsley et al. (1995) as part of their analysis of the MADS box (*AGL*) gene family in Arabidopsis. They have shown, using in situ hybridization, that *AGL15* mRNA starts to accumulate as early as the eight-cell stage in the Arabidopsis embryo proper.

An important feature of *AGL15* is that it belongs to a family of regulatory proteins that often play pivotal roles in developmental switching and differentiation processes. Processes regulated by MADS box genes include mating-type switching in yeast (*MCM1*; Herskowitz, 1989), myoblast differentiation in Drosophila and humans (*MEF2*; Kaushal et al., 1994; Lilly

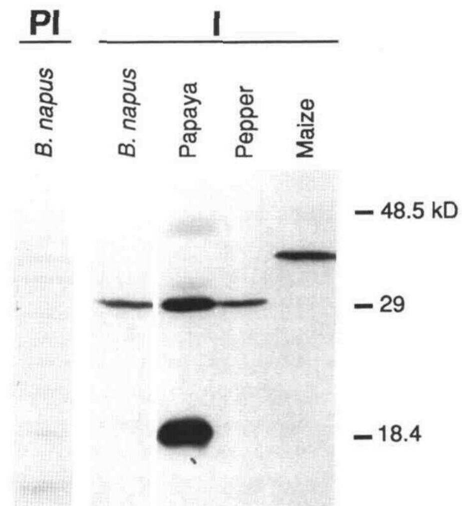


Figure 8. Detection of AGL15-Related Proteins in Embryos of Other Flowering Plant Families.

Shown are immunoblots of soluble protein extracts (150 μg per lane) from developing *B. napus*, papaya, and maize embryos or immature pepper seeds. The blots were incubated with preimmune serum (PI) or affinity-purified polyclonal antibodies (I) raised against the K box and C-terminal domains of AGL15-1. The mobility of the size standards is indicated at right.

et al., 1995), and floral meristem and organ identity specification in plants (e.g., *AG*, *AP1*, *AP3*, and *PI*; reviewed in Ma, 1994). Seventeen members of the MADS domain family have been characterized in Arabidopsis thus far (Rounsley et al., 1995), and based on low-stringency genomic DNA blot analysis (Ma et al., 1991), additional members may exist. Many of the family members are expressed in the context of floral development, but a function has been assigned to the gene product in only five cases. The analysis of individual genes may be complicated, in some cases, by family members with either partially or completely redundant functions. Phenotypes due to the disruption of the *CAL* locus, for instance, can be observed only when the *AP1* locus is also disrupted (Kempin et al., 1995), indicating that the functions of the *CAL* and *AP1* gene products are partially redundant. Other close "pairs," such as *AGL2* and *AGL4*, have been identified based on sequence and general expression pattern (Ma et al., 1991) and may also encode gene products with redundant functions. In contrast, *AGL15* has no close relatives within the MADS domain family of Arabidopsis. Although *AGL15* appears to be most closely related to *AP1*, the two sequences are different enough to be considered part of separate subgroups in the family (Rounsley et al., 1995). Therefore, we consider it likely that AGL15 has a unique function corresponding to its distinct expression pattern and amino acid sequence.

Similar to other MADS domain proteins, AGL15 appears to be present and conserved in members of many different angiosperm lineages. Genes homologous to the Arabidopsis *AG* gene have been isolated from Antirrhinum, tomato, maize, and several other plants, including some gymnosperms (Tandre et al., 1995). Despite the fact that floral morphology is extremely variable in angiosperms as a group, the AG protein sequences exhibit a high degree of conservation. Work in Antirrhinum and tomato indicates that the function of the *AG* gene product is often conserved between species as well (Bradley et al., 1993; Pnueli et al., 1994). Using antibodies raised against the K and C-terminal domains, we showed that proteins immunologically related to AGL15 are present in developing embryos of members of several dicot and one monocot family. This indicates that elements of AGL15 structure, in addition to the putative DNA binding MADS domain, are conserved and that at least some aspects of the developmental regulation of gene expression are conserved as well. Residues important to protein function are likely to be highly conserved, and as we characterize additional *AGL15* sequences, we expect to be able to define elements related to the AGL15 protein's ability to interact with other elements of the transcriptional complex.

The deduced amino acid sequences of the three *AGL15* genes that we isolated from members of a single family (Brassicaceae) are very similar overall (~78% identity comparing Arabidopsis AGL15 and *B. napus* AGL15-1 sequences). This is consistent with the results of previous studies comparing *B. napus* and Arabidopsis sequences for the MADS domain proteins AG (94% identity overall; Mandel et al., 1992a) and CAL (82% identity; Kempin et al., 1995). We isolated two *AGL15* sequences from the *B. napus* genome. These show approximately the same level of amino acid identity (83% overall). Considering the relatively recent origin of the *B. napus* genome from the putative diploid progenitors, *B. oleracea* and *B. rapa* (both of which have partially duplicated genomes; Lydiate et al., 1993), the existence of two and possibly more forms of *AGL15* with this degree of divergence is not surprising.

The temporal and spatial patterns of expression of *AGL15* are consistent with it being a factor involved in embryo specification. *AGL15* mRNA is present throughout embryo development and maturation. It is also present in all cells of the embryo proper. Therefore, a role in specification of particular tissues or elements of the basic embryo body plan seems unlikely. The pattern of expression would be consistent, however, with a more global regulatory role, such as a promoter of embryo-specific programs or inhibitor of postgermination programs. If this scenario is correct, functional homologs of *AGL15* should exist in all seed plants but would not be found in vascular plants like ferns, in which sporophyte development is basically continuous. It is also possible that AGL15 is not an important determinant of either spatial or temporal information by itself but rather interacts with another factor(s) that can impart this specificity. Examples of MADS domain proteins that are known to work in conjunction with other transcription factors include GLOBOSA and DEFICIENS (two florally expressed MADS domain proteins) in Antirrhinum (Schwarz-Sommer et al., 1992; Tröbner et al., 1992) and MCM1 (MADS domain protein) and α2 (homeodomain protein) in yeast (Herskowitz, 1989). MCM1 can also act alone or in conjunction with another factor, such as α1, depending on the mating type of the particular yeast cell (Herskowitz, 1989).

Experiments designed to determine whether AGL15 interacts with other factors to bind DNA are under way. To gain further insight into the role(s) AGL15 may play in development, we are currently generating transgenic plants with altered levels of AGL15 in embryonic and postgermination stages.

METHODS

Plant Material

Brassica napus (cv Tower) plants were grown to maturity in an environmental chamber (Conviron; Controlled Environments Inc., Pembina, ND) under a 16-hr-light (370 μE m^{-2} sec^{-1} at flower level, 15°C) and 8-hr-dark (10°C) regime. Flowers were hand pollinated on the day that they opened, and developing embryos were staged on the basis of morphology and/or chronological age (DAP, days after pollination). Tissue samples collected for RNA analyses were frozen in liquid N_2 immediately after excision and stored at −80°C. *Arabidopsis thaliana* ecotype Wassilewskija plants used for genomic DNA preparations were grown in sterile liquid culture (Reiter et al., 1992) under ambient room temperature and light conditions with continuous shaking.

cDNA and Genomic Libraries

To construct a cDNA library of sequences expressed in young embryos, *B. napus* seeds were opened at 16 to 19 DAP, and embryos at the transition between globular and heart stages were collected.

One microgram of poly(A)$^+$ RNA was isolated from ~8000 embryos, and cDNA was prepared and cloned using the λZAPII vector and cloning system (Stratagene). Approximately 1.5 x 10^6 recombinant phage were present in the unamplified transition-stage embryo library. Additional libraries were obtained from the laboratories of D. Meinke (Oklahoma State University, Stillwater, OK; Arabidopsis ecotype Wassilewskija silique cDNA library), D. Marks (University of Minnesota, St. Paul, MN; Arabidopsis ecotype Wassilewskija genomic library), and A. Huang (University of California, Riverside, CA; *B. napus* cv Bridger genomic library).

Nucleic Acid Isolation and Analysis

Differential display was performed as described by Liang and Pardee (1992). One microgram of total RNA from each sample (16-, 20-, and 45-DAP *B. napus* embryos as well as mature leaves and vegetative shoot apices [collected from 2-week-old seedlings]) was used in the first strand synthesis reaction. Polymerase chain reaction (PCR) was then performed with one-tenth of the first strand cDNA reaction, various primer sets, and ^{35}S-dATP in a 20-μL reaction. After 40 amplification cycles at 94°C for 30 sec, 42°C for 1 min, and 72°C for 30 sec, 4 μL of the reaction mixture was loaded onto a 6% polyacrylamide sequencing gel. Differential bands identified by autoradiography were then excised from the dried gel, rehydrated, and electroeluted before reamplification and cloning into pBluescript SK− (Stratagene), which was digested with EcoRV and tailed with a single thymidine residue using Taq polymerase.

Genomic DNA isolation and RNA and DNA gel blot analyses were performed as described by Heck et al. (1993) using either 5 μg of total RNA or 3 to 5 μg of genomic DNA per lane. Total RNA was isolated after extracting samples with hot SDS-phenol as described by Finkelstein et al. (1985), and poly(A)$^+$ RNA was prepared from total RNA using the Poly-A-Tract system (Promega). Slot blots were loaded with 1 μg of poly(A)$^+$ RNA or yeast tRNA per slot according to Sambrook et al. (1989) and hybridized under the same conditions as those used for DNA blot analyses. Four different probes were labeled with phosphorus-32 for hybridization and are as follows: probe 1 is the 759-bp product generated by PCR from Arabidopsis *AGL15* (for AGAMOUS-like) cDNA using oligonucleotides 5′-AGGAGGATTGTGCAGAG-3′ and 5′-CAAACTCTCAGCTAGGCA-3′. Probe 2 is the 563-bp cDNA generated by PCR amplification from *B. napus AGL15-1* cDNA using oligonucleotides 5′-GACTGTTTTGTCTCGATGC-3′ (primes within the second intron that is unspliced in one particular cDNA clone) and 5′-ACGGATCCTTTGTGCAGT-3′. *AGL2* was amplified from an Arabidopsis silique cDNA library and partially sequenced; the probe was made from the C-terminal EcoRI-EcoRI fragment shown to be specific for this gene (Flanagan and Ma, 1994). The EF-1α (translation elongation factor) probe was made from a PCR-derived portion of EF-1α–A1 from Arabidopsis (Curie et al., 1991). Final washes of blots were done under high-stringency conditions (0.1 × SSC [1 × SSC is 0.15 M NaCl, 0.015 M sodium citrate], 0.1% SDS at 65°C). Slot blots were quantitated with a Molecular Dynamics (Sunnyvale, CA) PhosphorImager 425e.

Libraries were screened under stringent conditions (0.5 × SSC, 0.1% SDS, 65°C final wash) using probes labeled with digoxigenin-derivatized dUTP. Positive plaques were visualized using the chemiluminescent-based Genius system (Boehringer Mannheim).

Selected differential display, cDNA, and genomic clones were chosen for further analysis. Sequencing was done on both strands using a Sequenase kit (U.S. Biochemical Corp.). Sequences were compiled, analyzed, and compared using the DNASTAR software package (DNASTAR, Inc., Madison, WI), the BLAST sequence alignment algorithm (Altschul et al., 1990), and data bases at the National Center for Biotechnology Information (Bethesda, MD).

Mapping of *AGL15*

AGL15 was mapped in Arabidopsis by following segregation of nucleotide polymorphisms in F_2 families from a cross between the Columbia and Landsberg *erecta* ecotypes. The third intron of *AGL15* was amplified from genomic DNA of each family and then sequenced directly. Genotype was scored by noting the single nucleotide transition and two transversions that occur within this sequence interval. Linkage to markers previously mapped in these families was established using the MAPMAKER computer package (Lander et al., 1987). Fine-scale mapping was done by R. Schmidt, K. Love, and C. Dean (Cambridge Laboratory, John Innes Center, Norwich, UK) using hybridization of *AGL15* to ordered yeast artificial chromosomes (YACs) spanning the top of chromosome 5.

In Situ Hybridizations

Embryos were processed for in situ hybridizations as described by Fernandez et al. (1991). ^{35}S-labeled RNA probes (~2 × 10^9 dpm/μg) were generated using pBluescribe and pBluescript vector systems (Stratagene), T3 RNA polymerase, and high specific activity ^{35}S-UTP (1000 to 1500 Ci/mmol; Du Pont–New England Nuclear). Templates were derived from cDNA sequences downstream of the region encoding the MADS domain: a 571-nucleotide antisense RNA probe (probe 3, corresponding to a PCR product generated with oligonucleotides 5′-ACGGATCCTTTGTGCAGT-3′ and 5′-TCAGAGCTCTTCAGATGTTC-3′) and a 421-nucleotide sense RNA probe (probe 4, a BglII-PstI fragment). Both probes were hydrolyzed to ~200 bp in length. Sections mounted on ProbeOn Plus glass slides (Fisher Scientific) were hybridized with the probe overnight at 42°C, then treated with RNase A, and washed at moderate stringency (0.1 × SSC at 55°C), as described by Cox et al. (1984). Slides were coated with autoradiography emulsion (type NTB2; Eastman Kodak Co., Rochester, NY) and exposed for 16 days at 4°C. Sections were counterstained with toluidine blue (0.05% [w/v] aqueous solution), and photomicrographs were taken using Kodak Pan X film and a Zeiss Akioskop (Oberkochen, Germany) equipped with a dark-field stop.

Antibody Production and Immunodetection of AGL15 in Plant Extracts

To produce AGL15 for use as an antigen, *B. napus AGL15-1*–specific oligonucleotides that were flanked by NcoI and BamHI restriction sites and incorporated a termination codon were used to amplify the *AGL15-1* nucleotide sequence downstream of the MADS domain from the *B. napus* transition-stage embryo cDNA library. The PCR product (sequence corresponding to amino acid residues 62 to 258) was cloned into expression vector pET-15b (Novagen, Madison, WI). Truncated AGL15-1 was overexpressed by transformation of the expression host BL21(DE3) and induction with 1 mM isopropyl β-D-thiogalactopyranoside as described by Perry and Keegstra (1994). Inclusion bodies were isolated, and the inclusion protein was solubilized in 8 M urea and 10 mM β-mercaptoethanol in 50 mM Tris-HCl, 5 mM $MgCl_2$, pH 7.6, for 5 min at room temperature. Solubilized protein was further purified by electrophoresis on two successive preparative ProSieve agarose gels (FMC, Rockland, ME). The protein band corresponding to truncated AGL15-1 was excised from the gel and used to immunize rabbits

at the University of Wisconsin–Madison Medical School Animal Care Unit. Antibodies that recognized truncated AGL15-1 were affinity purified from antisera as described by Tang (1993).

To prepare protein extracts, embryos were dissected from developing seeds of *B. napus* cv Tower (25 DAP), *Carica papaya* (cotyledon-stage embryos, 3 to 4 mm length), and *Zea mays* W64A × W438 (18 DAP). Whole seed with torpedo-stage embryos were collected from pepper (chili) plants (*Capsicum annuum*). Frozen tissue was ground and homogenized in ice-cold extraction buffer (100 mM Tris, pH 7.5, 10% sucrose, 5 mM EDTA, 5 mM EGTA, 1 mM phenylmethylsulfonyl fluoride, 10 μg/mL leupeptin, 10 μg/mL pepstatin A) using 1 to 2 mL/g of tissue. Insoluble protein was removed by centrifugation at 16,000*g* for 2 min, and the remaining supernatant was boiled in an equal volume of 2 × SDS sample buffer (0.125 M Tris-HCl, 4% SDS, 20% glycerol, 0.2 M DTT, 0.02% bromophenol blue, pH 6.8) for 4 min. Soluble proteins (150 μg per lane) were separated on a denaturing 15% polyacrylamide gel before blotting onto an Immobilon polyvinylidene fluoride membrane (Millipore, Bedford, MA). Blots were incubated either with preimmune sera diluted 1:500 or antibodies affinity purified against truncated AGL15-1 (final dilution ~1:1500 relative to original immune serum). The immunoreactive protein was visualized using the Lumi-GLO system (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, MD) with the secondary antibody diluted 1:2000. The blot was exposed to x-ray film (Kodak XAR5) for 1 to 3 min.

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REFERENCES

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., and Lipman, D.J. (1990). Basic local alignment search tool. J. Mol. Biol. **215,** 403–410.

Baumlein, H., Miséra, S., Kolle, K., Horstmann, C., Wobus, U., and Miller, A.J. (1994). The *fus3* gene of *Arabidopsis thaliana* is a regulator of gene expression during late embryogenesis. Plant J. **6,** 379–387.

Bradley, D., Carpenter, R., Sommer, H., Hartley, N., and Coen, E. (1993). Complementary floral homeotic phenotypes result from opposite orientations of a transposon at the *plena* locus of Antirrhinum. Cell **72,** 85–95.

Castle, L.A., Errampalli, D., Atherton, T.L., Franzmann, L.H., Yoon, E.S., and Meinke, D.W. (1993). Genetic and molecular characterization of embryonic mutants identified following seed transformation in *Arabidopsis*. Mol. Gen. Genet. **241,** 504–514.

Cox, K.H., DeLeon, D.V., and Angerer, L.M. (1984). Detection of mRNAs in sea urchin embryos by *in situ* hybridization using asymmetric RNA probes. Dev. Biol. **101,** 485–502.

Curie, C., Liboz, T., Bardet, C., Gander, E., Medale, C., Axelos, M., and Lescure, B. (1991). *cis*- and *trans*-acting elements involved in the activation of *Arabidopsis thaliana* A1 gene encoding the translation elongation factor EF-1α. Nucleic Acids Res. **19,** 1305–1310.

Dubois, E., Bercy, J., and Messenguy, F. (1987). Characterization of two genes, *ARGI* and *ARGIII*, required for specific regulation of the arginine metabolism in yeast. Mol. Gen. Genet. **207,** 142–148.

Fernandez, D.E., Turner, F.R., and Crouch, M.L. (1991). *In situ* localization of storage protein mRNAs in developing meristems of *Brassica napus* embryos. Development **111,** 299–313.

Finkelstein, R.R., Tenbarge, K.M., Shumway, J.E., and Crouch, M.L. (1985). Role of ABA in maturation of rapeseed embryos. Plant Physiol. **78,** 630–636.

Flanagan, C.A., and Ma, H. (1994). Spatially and temporally regulated expression of the MADS-box gene *AGL2* in wild-type and mutant *Arabidopsis* flowers. Plant Mol. Biol. **26,** 581–595.

Franzmann, L.H., Yoon, E.S., and Meinke, D.W. (1995). Saturating the genetic map of *Arabidopsis thaliana* with embryonic mutations. Plant J. **7,** 341–350.

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

Goto, K., and Meyerowitz, E.M. (1994). Function and regulation of the *Arabidopsis* floral homeotic gene *PISTILLATA*. Genes Dev. **8,** 1548–1560.

Hauge, B.M., Hanley, S.M., Cartinhour, S., Cherry, J.M., Goodman, H.M., Koornneef, M., Stam, P., Chang, C., Kempin, S., Medrano, L., and Meyerowitz, E.M. (1993). An integrated genetic/RFLP map of the *Arabidopsis thaliana* genome. Plant J. **3,** 745–754.

Heck, G.R., Chamberlain, A.K., and Ho, T.-H.D. (1993). Barley embryo globulin 1 gene, *Beg1*: Characterization of cDNA, chromosome mapping and regulation of expression. Mol. Gen. Genet. **239,** 209–218.

Herskowitz, I. (1989). A regulatory hierarchy for cell specialization in yeast. Nature **342,** 749–757.

Huijser, P., Klein, J., Wolf-Ekkehard, L., Meijer, H., Saedler, H., and Sommer, H. (1992). Bracteomania, an inflorescence anomaly, is caused by the loss of function of the MADS-box gene *squamosa* in *Antirrhinum majus*. EMBO J. **11,** 1239–1249.

Jack, T., Brockman, L.L., and Meyerowitz, E.M. (1992). The homeotic gene *APETALA3* of *Arabidopsis thaliana* encodes a MADS box and is expressed in petals and stamens. Cell **68,** 683–697.

Kaushal, S., Schneider, J.W., Nadal-Ginard, B., and Mahdavi, V. (1994). Activation of the myogenic lineage by MEF2A, a factor that induces and cooperates with MyoD. Science **266**, 1236–1240.

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.

Kempin, S.A., Savidge, B., and Yanofsky, M.F. (1995). Molecular basis for the *cauliflower* phenotype in *Arabidopsis*. Science **267**, 522–525.

Lander, E.S., Green, P., Abrahamson, J., Barlow, A., Daly, M.J., Lincoln, S.E., and Newburg, L. (1987). MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics **1**, 174–181.

Liang, P., and Pardee, A.B. (1992). Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. Science **257**, 967–971.

Lilly, B., Zhoa, B., Ranganayakulu, R., Paterson, B.M., Schulz, R.A., and Olson, E.N. (1995). Requirement of MADS domain transcription factor D-MEF2 for muscle formation in *Drosophila*. Science **267**, 688–693.

Lindsey, K., and Topping, J.F. (1993). Embryogenesis: A question of pattern. J. Exp. Bot. **44**, 359–374.

Lydiate, D., Sharpe, A., Lagercrantz, U., and Parkin, I. (1993). Mapping the *Brassica* genome. Outlook Agric. **22**, 85–92.

Ma, H. (1994). The unfolding drama of flower development: Recent results from genetic and molecular analyses. Genes Dev. **8**, 745–756.

Ma, H., Yanofsky, M.F., and Meyerowitz, E.M. (1991). *AGL1-AGL6*, an *Arabidopsis* gene family with similarity to floral homeotic and transcription factor genes. Genes Dev. **5**, 484–495.

Mandel, M.A., Bowman, J.L., Kempin, S.A., Ma, H., Meyerowitz, E.M., and Yanofsky, M.F. (1992a). Manipulation of flower structure in transgenic tobacco. Cell **71**, 133–143.

Mandel, M.A., Gustafson-Brown, C., Savidge, B., and Yanofsky, M.F. (1992b). Molecular characterization of the *Arabidopsis* floral homeotic gene *APETALA1*. Nature **360**, 273–277.

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

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

Nambara, E., Keith, K., McCourt, P., and Naito, S. (1995). A regulatory role for the *ABI3* gene in the establishment of embryo maturation in *Arabidopsis thaliana*. Development **121**, 629–636.

Norman, C., Runswick, M., Pollock, R., and Treisman, R. (1988). Isolation and properties of cDNA clones encoding SRF, a transcription factor that binds to the c-*fos* serum response element. Cell **55**, 989–1003.

Passmore, S., Maine, G.T., Elble, R., Christ, C., and Tye, B.K. (1988). A *Saccharomyces cerevisiae* protein involved in plasmid maintenance is necessary for mating in MATα cells. J. Mol. Biol. **204**, 593–606.

Perry, S.E., and Keegstra, K. (1994). Envelope membrane proteins that interact with chloroplastic precursor proteins. Plant Cell **6**, 93–105.

Pnueli, L., Abu-Abeid, M., Zamir, D., Nacken, W., Schwarz-Sommer, Z., and Lifschitz, E. (1991). The MADS box gene family in tomato: Temporal expression during floral development, conserved secondary structures and homology with homeotic genes from *Antirrhinum* and *Arabidopsis*. Plant J. **1**, 255–266.

Pneuli, L., Hareven, D., Rounsley, S.D., Yanofsky, M.F., and Lifschitz, E. (1994). Isolation of the tomato *AGAMOUS* gene *TAG1* and analysis of its homeotic role in transgenic plants. Plant Cell **6**, 163–173.

Putterill, J., Robson, F., Lee, K., and Coupland, G. (1993). Chromosome walking with YAC clones in *Arabidopsis*: Isolation of 1700 kb of contiguous DNA on chromosome 5, including a 300 kb region containing the flowering-time gene *CO*. Mol. Gen. Genet. **239**, 145–157.

Reiter, R.S., Young, R.M., and Scolnik, P.A. (1992). Genetic linkage of the *Arabidopsis* genome: Methods for mapping with recombinant inbreds and random amplified polymorphic DNAs (RAPDs). In Methods in Arabidopsis Research, C. Koncz, N.-H. Chua, and J. Schell, eds (River Edge, NJ: World Scientific Publishing Co.), pp. 170–190.

Rounsley, S.D., Ditta, G.S., and Yanofsky, M.F. (1995). Diverse roles for MADS box genes in Arabidopsis development. Plant Cell **7**, 1259–1269.

Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual. (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press).

Schneider, T., Dinkins, R., Robinson, K., Shellhammer, J., and Meinke, D.W. (1989). An embryo-lethal mutant of *Arabidopsis thaliana* is a biotin auxotroph. Dev. Biol. **131**, 161–167.

Schwarz-Sommer, Z., Huijser, P., Nacken, W., Saedler, H., and Sommer, H. (1990). Genetic control of flower development by homeotic genes in *Antirrhinum majus*. Science **250**, 931–936.

Schwarz-Sommer, Z., Hue, I., Huijser, P., Flor, P.J., Hansen, R., Tetens, F., Lönnig, W.-E., Saedler, H., and Sommer, H. (1992). Characterization of the *Antirrhinum* floral homeotic MADS-box gene *deficiens*: Evidence for DNA binding and autoregulation of its persistent expression throughout flower development. EMBO J. **11**, 251–263.

Scott, R., Dagless, E., Hodge, R., Paul, W., Soufleri, I., and Draper, J. (1991). Patterns of gene expression in developing anthers of *Brassica napus*. Plant Mol. Biol. **17**, 195–207.

Sheridan, W.F., and Clark, J.K. (1993). Mutational analysis of morphogenesis of the maize embryo. Plant J. **3**, 347–358.

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

Sommer, H., Beltrán, J.-P., Huijser, P., Pape, H., Lönnig, W., Saedler, H., and Schwarz-Sommer, Z. (1990). *Deficiens*, a homeotic gene involved in the control of flower morphogenesis in *Antirrhinum majus*: The protein shows homology to transcription factors. EMBO J. **9**, 605–613.

Tandre, K., Albert, V.A., Sundås, A., and Engström, P. (1995). Conifer homologues to genes that control floral development in angiosperms. Plant Mol. Biol. **27**, 69–78.

Tang, W.-J.Y. (1993). Blot-affinity purification of antibodies. In Methods in Cell Biology, Vol. 37, Antibodies in Cell Biology, D.J. Asai, ed (San Diego, CA: Academic Press), pp. 95–104.

Tröbner, W., Ramirez, L., Motte, P., Hue, I., Huijser, P., Lönnig, W.-E., Saedler, H., Sommer, H., and Schwarz-Sommer, Z. (1992). *GLOBOSA*: A homeotic gene which interacts with *DEFICIENS* in the control of *Antirrhinum* floral organogenesis. EMBO J. **11**, 4693–4704.

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

West, M.A.L., Yee, K.M., 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.

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.

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