Glossy15, an *APETALA2*-like gene from maize that regulates leaf epidermal cell identity

Stephen P. Moose[1,2,4] and Paul H. Sisco[2,3,5]

Departments of [1]Genetics and [2]Crop Science and [3]United States Department of Agriculture/Agricultural Research Service, North Carolina State University, Raleigh, North Carolina 27695-7620 USA

Vegetative development in many plants progresses through distinct juvenile and adult phases. In maize, the transition from juvenile to adult shoot development affects a variety of leaf epidermal cell traits. These include epicuticular waxes, leaf hairs, and cell wall characteristics. Previous genetic and phenotypic analyses have shown that the maize *Glossy15* (*Gl15*) gene is required for the expression of juvenile epidermal traits after leaf 2. We report here the molecular cloning of the *Gl15* gene using a *defective Suppressor-Mutator* (*dSpm*) element insertion as a transposon-tag. Consistent with the *gl15* mutant phenotype, the pattern of *Gl15* mRNA expression was correlated with a juvenile leaf epidermal cell identity and was regulated by upstream factors such as *Corngrass1*. The *Gl15* gene encodes a putative transcription factor with significant sequence similarity to the *Arabidopsis* regulatory genes *APETALA2* and *AINTEGUMENTA*, which act primarily to regulate floral organ identity and ovule development. This finding expands the known functions of *APETALA2*-related genes to include the control of both vegetative and reproductive lateral organ identity and provides molecular support for the hypothesis that leaves and floral organs are related structures derived from a common growth plan.

[*Key Words*: Leaf development; organ identity; heteroblasty; phase change; transposon-tagging]

Received August 12, 1996; revised version accepted October 16, 1996.

Vegetative shoot development in many plants is characterized by the production of both juvenile and adult leaves, or heteroblasty (Goebel 1900; Allsopp 1967). The basal juvenile and upper adult leaves are often sufficiently distinct with respect to their shape, anatomy, and physiological characteristics that they may be considered to have different identities in the same sense that floral organs such as sepals and petals have different identities (Poethig 1990; Smith and Hake 1992). The determination of juvenile and adult leaf characteristics is subject to both genetic and hormonal influences that act on the shoot apical meristem and developing leaf primordia (for review, see Lawson and Poethig 1995). Currently, little is known about the molecular mechanisms that regulate the transition from juvenile to adult shoot development.

The juvenile and adult leaves of maize differ for a large set of epidermal traits (Poethig 1990). Some of these are illustrated in Figure 1, and include the formation of specialized cell types such as leaf hairs and bulliform cells, the ultrastructure and biochemical composition of epicuticular waxes, epidermal cell morphology, and cell wall reactions with histochemical stains. The epidermis of the basal 5 or 6 juvenile leaves consists largely of a single cell type that has acidic cell walls and produces juvenile waxes. Beginning with leaf 6 there is a transition to the adult leaf form such that beyond node 8, leaves are exclusively adult. The adult leaf epidermis develops a number of differentiated cell types. Bulliform cells and their associated leaf hairs occur in longitudinal files over leaf veins and are separated by cells that lack juvenile waxes and have invaginated, neutral cell walls.

A number of maize mutations regulate the spatial/temporal expression of juvenile and adult leaf epidermal traits during shoot development. The dominant, gain-of-function *Corngrass1* (*Cg1*), *Teopod1* (*Tp1*), and *Teopod2* (*Tp2*) mutations extend the expression of all juvenile vegetative traits (axillary tillers, adventitious roots, juvenile leaves), leading to an increase in the number of juvenile leaves or leaves with both juvenile and adult characteristics (Galinat 1966; Poethig 1988). The maize *dwarf* mutants, which affect gibberellic acid biosynthesis and have a short andromonecious phenotype, also produce more juvenile leaves and interact synergistically with the *Tp* mutations (Evans and Poethig 1995). In contrast to the pleiotropic effects on vegetative and reproductive growth associated with each of these mutations, recessive *glossy15* (*gl15*) mutations condition the re-

[4]Current address: Department of Biology, University of California–San Diego, La Jolla, CA 92093-0116.
[5]Corresponding author.

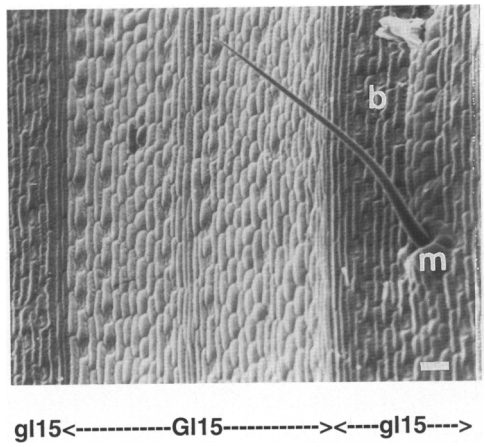

Figure 1. The *glossy15-mutable* phenotype. A scanning electron micrograph of the adaxial surface of leaf 4 from an *Spm*-active, homozygous *gl15-mutable* plant. A revertant sector (*center*), in which *Gl15* activity has been restored by the transposition of the *dSpm* element from the *gl15-m1* allele, is flanked by *gl15* mutant tissue, where the *dSpm* insertion disrupts gene function. The juvenile epidermal cells within the *Gl15*-active sector produce a visible layer of crystalline waxes and possess wavy lateral cell walls. In the adjacent *gl15* mutant tissues, juvenile cells are replaced by adult epidermal cells with invaginated walls, bulliform cells (b), and leaf macrohairs (m). Bar, 100 μm.

placement of juvenile with adult leaf epidermal traits beginning with the third leaf but do not affect any other phenotypes associated with the transition from juvenile to adult vegetative growth (Evans et al. 1994; Moose and Sisco 1994). Additionally, genetic analysis has demonstrated that *gl15* acts downstream of the *Cg1*, *Tp1*, *Tp2*, *dwarf1*, and *dwarf3* mutations (Moose and Sisco 1994; Evans and Poethig 1995). These observations suggest that *Gl15* acts specifically to promote a juvenile leaf epidermal cell identity.

We have characterized previously the phenotypic effects associated with a transposon-induced mutable allele of *gl15* (*gl15-m1*) caused by the insertion of a *defective Suppressor-Mutator* (*dSpm*) element (Moose and Sisco 1994). *Gl15* acts in a cell-autonomous fashion and is required for the coordinate activation of juvenile and suppression of adult traits in the leaf epidermis (Fig. 1). Here we report the molecular isolation of the *Gl15* gene using the *dSpm* insertion as a transposon-tag. *Gl15* mRNA expression is correlated with a juvenile leaf identity beyond leaf 2. The predicted *Gl15* gene product encodes a member of the AP2-domain family, based on its significant similarities to the two AP2 domains from the *Arabidopsis* regulatory genes *APETALA2* (*AP2*; Jofuku et al. 1994) and *AINTEGUMENTA* (*ANT*; Elliott et al. 1996; Klucher et al. 1996). Sequences related specifically to *Gl15* are also present in other monocot and dicot plant species. The similarities between *Gl15* and genes regulating floral organ identity (*AP2*) and ovule development (*AP2*, *ANT*) indicate that *AP2*-related genes participate

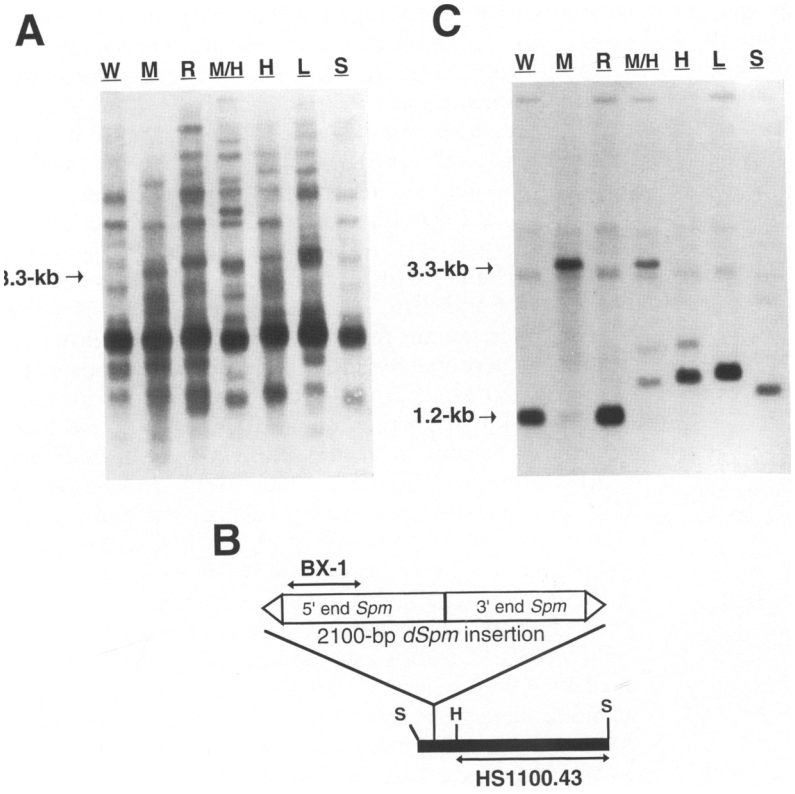

Figure 2. Molecular cloning of *Gl15*. (*A*) DNA gel blot of *Sst*I-digested genomic DNAs from plants carrying different alleles of the *Gl15* locus probed with the BX-1 fragment of the Spm transposon. Alleles are designated as follows: (W) normal *Gl15-W64A* allele from the inbred W64A; (M) *gl15-m1::dSpm*; (R) germinal revertant derivative obtained from *gl15-m1*. Three known *gl15-Ref* alleles are designated: (H) Hayes; (L) Lambert; and (S) Sprague (Moose and Sisco 1994). The M/H lane contained DNA from a sibling of the germinal revertant plant. This sibling was heterozygous for *gl15-m1* and *gl15-H* and showed a *gl15-mutable* phenotype. A 3.3-kb band in plants carrying the *gl15-m1* allele and absent from revertant derivatives of *gl15-m1* is indicated by the arrow. (*B*) Restriction map of the cloned 3.3-kb *Sst*I fragment from the *gl15-m1* allele. The 2.1-kb *dSpm* element insertion and the relative position of the BX-1 fragment used as a hybridization probe are shown. The region spanned by the HS1100.43 probe flanking the *dSpm* insertion is underlined. (*C*) The blot in *A* was stripped and rehybridized with the HS1100.43 probe derived from the cloned 3.3-kb fragment in *B*. The 3.3-kb fragment from the *gl15-m1* allele and the 1.2-kb fragment from the *Gl15-W64A* and either somatic or germinal revertant alleles are indicated. Distinct RFLPs relative to wild type were also observed among the three *gl15-Ref* alleles.

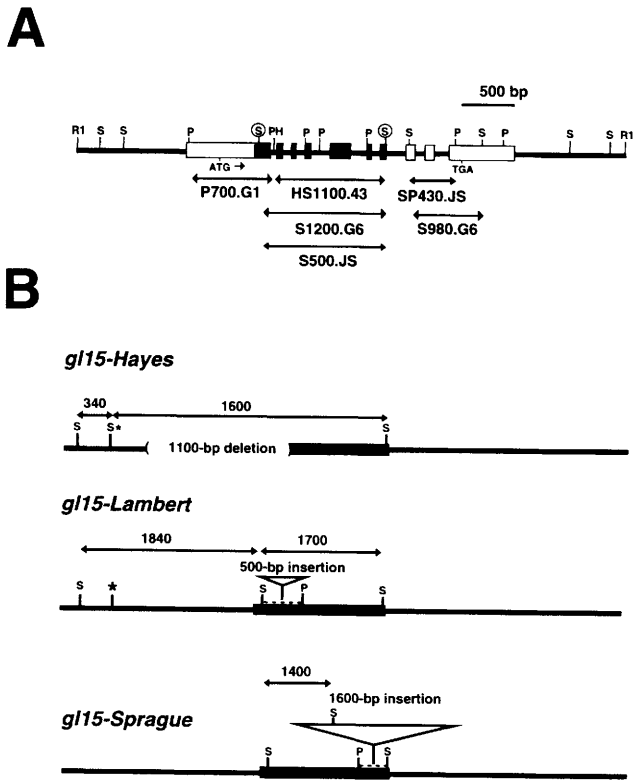

Figure 3. Structure of wild-type and mutant *Gl15* alleles. (*A*) Structure of the 6.0-kb *Eco*RI restriction fragment from the *Gl15-W64A* allele. *Eco*RI (RI), *Sst*I (S), and *Pst*I (P) restriction sites are indicated, the two *Sst*I sites delimiting the 3.3-kb fragment initially isolated from the *gl15-m1::dSpm* allele are circled. The black box represents the putative nuclear localization signal and two AP2 domains conserved between *Gl15* and *AP2*. The positions of the putative translational start (ATG) and stop (TAG) codons are labeled. The regions represented by the genomic (HS1100.43, P700.G1, S1200.G6, S980.G6) and cDNA (S500.JS, SP430.JS) restriction fragments used as hybridization probes are underlined. (*B*) Restriction maps of the *gl15-Ref* alleles for the region represented by the 6.0-kb *Eco*RI fragment from *Gl15-W64A*, derived from restriction and hybridization analysis of genomic DNA using the P700.G1, S1200.G6, and S980.G6 restriction fragments as probes. Only relevant *Sst*I (S) and *Pst*I (P) restriction sites are labeled for reference. For each map, the black box indicates the AP2 domain region conserved between *Gl15* and *AP2*. An asterisk represents the missing *Sst*I site in the *gl15-L* allele. This same site appears to be methyated partially in the *gl15-H* allele, denoted by an S-. The approximate positions and sizes of the lesions in the *gl15-Ref* mutant alleles relative to the *Gl15-W64A* allele are indicated by triangles (insertion) or parentheses (deletion). Dashed lines indicate the insertion lies within the two flanking restriction sites. Arrows above each map and the corresponding numbers indicate the sizes of fragments obtained for *Sst*I digests of genomic DNAs from each allele.

in the control of both vegetative and reproductive lateral organ identity in plants.

Results

Molecular cloning of Glossy15

A mutable allele of *Gl15* (*gl15-m1*) was isolated previously and determined by genetic tests to be induced by a *dSpm* transposable element insertion (Moose and Sisco 1994). The *dSpm* element fragment BX-1 (Cone et al. 1986) was used as a hybridization probe in DNA gel-blot analyses of families segregating for *gl15-m1*. We identified a 3.3-kb *Sst*I fragment that cosegregated with the *gl15-m1* mutant phenotype. Representative homozygous and heterozygous *gl15-m1* plants with the 3.3-kb *Spm*-hybridizing fragment are shown in Figure 2A (lanes M and M/H). Convincing evidence that the 3.3-kb *Sst*I fragment represented a *dSpm* insertion in the *Gl15* gene came from the absence of this fragment in DNA from plants homozygous for germinal revertant alleles derived from *gl15-m1* (Fig. 2A, lane R). No other *Spm*-hybridizing fragments from *gl15-m1* plants were found to be missing consistently from these derivatives.

A size-fractionated subgenomic library of *Sst*I fragments from homozygous *gl15-m1* plants was constructed and the 3.3-kb *Sst*I fragment isolated using the BX-1 probe. Figure 2B shows the restriction map of the cloned 3.3-kb *Sst*I fragment, which contained a 2.1-kb *dSpm* element insertion flanked by non-*Spm* sequences. A restriction fragment containing only non-*Spm* flanking DNA (HS1100.43) was used as a hybridization probe to the same DNA gel blot shown in Figure 2A. This blot contained DNAs representative of normal *Gl15* (inbred W64A) and three previously identified *gl15-Ref* mutant alleles (Moose and Sisco 1994) in addition to *gl15-m1*, a derivative revertant allele, and one of its mutant siblings. The resulting hybridization pattern is shown in Figure 2C. Homozygous *gl15-m1* plants, which exhibit an unstable phenotype in the presence of autonomous *Spm*, showed the expected 3.3-kb *Sst*I fragment and a low-intensity 1.2-kb fragment (lane M). The 1.2-kb fragment was correlated with the generation of somatic revertant sectors from *gl15-m1* and thus represented the original progenitor allele. Proof that the HS1100.43 fragment represented part of the *Gl15* gene came from comparing a germinal revertant derivative of *gl15-m1* (lane R) with its mutable sibling (lane M/H). The homozygous revertant plant contained only a 1.2-kb *Sst*I hybridizing fragment, whereas its mutable sibling, heterozygous for the *gl15-m1* and *Hayes* alleles, contained 3.3-kb (M) and 1.6-kb (H) fragments. The size difference between the restriction fragments representing *gl15-m1* and either its somatic or germinal revertant derivatives was consistent with the 2.1-kb *dSpm* insertion observed within the cloned 3.3-kb *Sst*I fragment (Fig. 2B). The 1.2-kb *Sst*I fragment was present in all 15 maize inbreds tested that carried normal *Gl15* alleles, such as the inbred W64A (Fig. 2C, lane W). In contrast, each of the three *gl15-Ref(erence)* alleles—*gl15-H(ayes)*, *gl15-L(ambert)*, and *gl15-S(prague)*—were associated with other fragment sizes (lanes H, L, and S).

The HS1100.43 fragment was used as a probe to isolate wild-type *Gl15* cDNA and genomic clones from the inbred W64A. Restriction mapping and DNA sequencing of these clones indicated that a 6.0-kb *Eco*RI genomic fragment contained the entire *Gl15* transcribed region, as shown in Figure 3A. To determine where the lesions

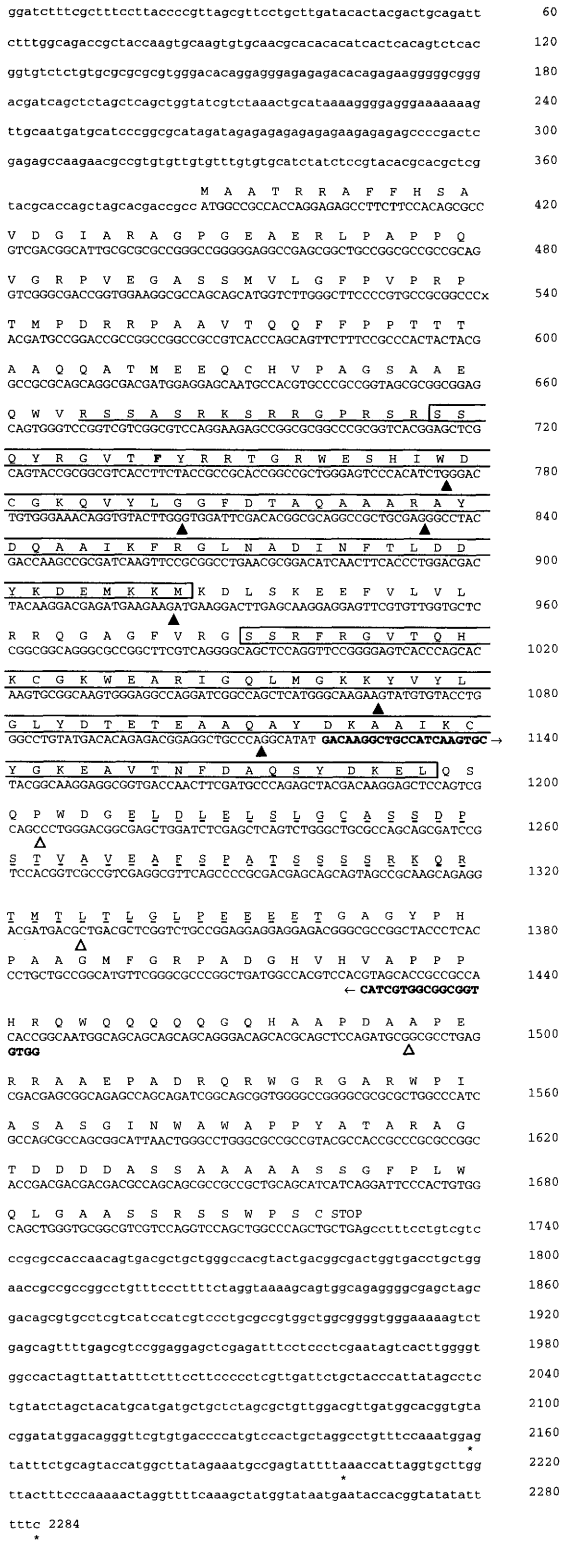

Figure 4. Sequence of the *Gl15* cDNA. The nucleotide sequence of the *Gl15* gene and the predicted amino-acid sequence of its corresponding protein are shown. Amino-acid numbers are given on the *left* and nucleotides are numbered on the *right* of the sequence. Boxed amino acids represent the two AP2 domains (Jofuku et al. 1994). The solid underlined residues indicate the basic region with potential nuclear-localization activity (Varagona et al. 1992), and the dashed underlined residues represent a serine-rich acidic domain. The six intron positions conserved between *Gl15* and *AP2* are marked by filled arrowheads, and open arrowheads show the positions of other introns. Asterisks denote the three observed polyadenylation sites. The phenylalanine (F) residue in bold indicates the position of the *dSpm* insertion in *gl15-m1*. Nucleotides in bold represent the sequences of the *Gl15* primers used in RT–PCR.

responsible for the other three known *gl15* mutations were located, DNA gel blots were prepared from plants homozygous for these alleles and hybridized sequentially to the P700.G1, HS1100.43, and S980.G6 probes. Analysis with multiple restriction enzymes demonstrated that each *gl15-Ref* allele exhibited either large insertions or deletions within the 1.2-kb *Sst*I fragment observed in wild-type alleles. The approximate sizes and locations of these lesions appear in Figure 3B. The *gl15-H* allele was characterized by a 1100-bp deletion that removed the entire 5′ end of the *Gl15* mRNA. The *gl15-L* allele contained a 500-bp insertion and the *gl15-S* allele a 1600-bp insertion with internal *Sst*I, *Bgl*II, *Hin*dIII, *Eco*RV, and *Eco*RI restriction sites. The nature of the insertions in the *gl15-L* and *gl15-S* alleles is currently unknown. An additional RFLP was identified in the *gl15-L* allele by the lack of one of the sites defining the 1500-bp *Sst*I fragment in the *Gl15*-W64A allele. This same site may also be only partially cleaved in the *gl15-H* allele, which would account for the two hybridizing fragments of 1.6-kb and 1.9-kb when HS1100.43 was used as a probe (Fig. 2C).

Gl15 *encodes an AP2-domain protein*

Figure 4 shows the complete nucleotide and predicted amino acid sequence of the longest (2284-bp) *Gl15* cDNA. Two unspliced introns, two differentially spliced introns, and three alternative polyadenylation sites were observed among the three *Gl15* cDNA clones that we sequenced. Variation in intron splicing is commonly observed in plant cDNA sequences (Nash and Walbot 1992). The longest (1341-bp) open reading frame encoded a 446-amino-acid polypeptide with a predicted molecular mass of 48 kD. Translation of the cDNA sequence upstream of the ATG start codon shown in Figure 4 revealed two short (five- to seven-amino-acid) open reading frames beginning at positions 246 and 249.

Data-base comparisons showed that the central portion of the predicted *Gl15* protein was very similar to the AP2 domain, a conserved motif first identified in the *Arabidopsis* floral homeotic gene *AP2*, but also found within a number of genes from *Arabidopsis*, rice, and other taxonomically diverse plant species (Jofuku et al. 1994; Weigel 1995). One of these proteins, EREBP-2, has been reported to possess sequence-specific DNA-binding activity, with the AP2 domain being essential for this activity (Ohme-Takagi and Shinshi 1995). Figure 5

shows the two AP2 domains from *Gl15* aligned with those of other genes present in sequence data bases. When compared with these other sequences, *Gl15* was most similar to *AP2*. Over a 170-amino-acid conserved region, *Gl15* and *AP2* shared 80% amino-acid identity. This conserved region spanned the two repeated AP2 domains, the linker region between the domains (Klucher et al. 1996) and a basic region representing a putative nuclear localization signal (Varagona et al. 1992). Additionally, within the AP2 domains, the positions of six introns were conserved exactly between *AP2* and *Gl15* (Fig. 4). Outside of the AP2 domains and the basic region, *Gl15* did not share any significant similarities in primary amino acid sequence with other genes. Like *AP2* and *ANT*, *Gl15* has a serine-rich acidic domain (amino acids 278–326) and a histidine/glutamine-rich region (residues 345–364) that may serve as transcription activation domains (Mitchell and Tjian 1989; Jofuku et al. 1994). However, in the *AP2* and *ANT* proteins, these putative transcription activation domains are in the amino-terminal region, whereas in *Gl15* they reside on the carboxy-terminal side.

The insertions or deletions associated with each of the characterized *gl15* mutant alleles were localized to the region encoding the conserved AP2 domains (Figs. 2C,3B). The insertion site of the *dSpm* transposable element in *gl15-m1* was near the 3′ end of the first exon. The deletion in *gl15-H* removed portions of the putative promoter region and much of the first exon, which resulted in a transcriptional null allele (see Fig. 7A, lane H, below). The relatively large insertions in the *gl15-L* and *gl15-S* mutant alleles also interrupt AP2 domains. Therefore, it is unlikely that any of the characterized *gl15* mutant alleles produce a functional protein.

Gl15, although very similar to *AP2*, is probably not the *AP2* ortholog in maize. Mutations in the two genes have very different phenotypes, and maize cDNA libraries probed with *AP2* have identified other *AP2*-related genes but not *Gl15* (G. Chuck and S. Hake, pers. comm.). At least one of these maize genes was more homologous to *AP2* than is *Gl15*. Another indication that *Gl15* represents a divergent member of the AP2-domain gene family was our finding that the *Gl15* S500.JS cDNA probe, which includes almost the entire conserved AP2 region, did not identify any hybridizing fragments in maize other than *Gl15* at standard stringencies. Moreover, DNA blots probed with *Gl15* subclones from both within and outside the conserved AP2 region revealed that sequences specifically related to *Gl15* are present as a single copy in several grasses (sorghum, pearl millet, *Andropogon virginicus*, and *Lolium*) and in low copy number in the dicot species soybean, tobacco, *Arabidopsis*, and peach (data not shown).

Gl15 *mRNA expression is correlated with the* gl15 *mutant phenotype*

The P700.G1 fragment was used in gel-blot analyses of maize leaf RNAs. Figure 6 shows a 2.2-kb *Gl15* mRNA whose size was in agreement with that of the longest *Gl15* cDNAs we isolated. The expression pattern of the *Gl15* mRNA was correlated with the *Gl15* phenotype. The *Gl15* mRNA was present as a low-abundance transcript in poly(A)$^+$ RNA from juvenile leaves 4–6. Hybridization to the P700.G1 probe was also detectable, but greatly reduced, in mRNA from leaves 1 and 2, whose juvenile phenotypes are not affected by *gl15* mutations. Because adult epidermal cell types are observed occasionally at the base of leaf 2 in *gl15* mutant plants (Evans

```
                    ___NLS?___=========================AP2  DOMAIN  1=========================
GLOSSY15        101 RKSRRGPRSRSSQYRGVTFYRRTGRWESHIWDC.........GKQVYLGGFDTAQAAARAYDQAAIKFRGVNADINFTLDDYLDEM 177
APETALA2        119 K*******************************.........***********H*******R********E*****NI***D*DL 195
RICE EST
AINTEGUMENTA    281 ***IDTFGQ*T*******RH*W***Y*A*L**NSFKKEGHSRK*R******Y*MEEK******L**L*YW*PSTHT**SAEN*QK*I 368
MAIZE ORF       160 ASGPPRRSASAHPSTVASPGTGGRGDMRRTCGNSCRREGQSRK*R******Y*KEEK******L**L**W*PTTTT**QVSN*EK*L 214

                    ________LINKER_____=========================AP2  DOMAIN  2=========================
GLOSSY15        178 KKMKDLSKEEFVLVLRRQGAGFVRGSSRFRGVTQH.KCGKWEARIGQLMGKKY.VYLGLYDTETEAAQAYDKAAIKCYGKEAVTNFDAQSYDKEL 270
APETALA2        196 *Q*TN*T*****H*****ST**P****KY****L*.***R****M**FL****.*********V***R*********N**D******PSI**E** 288
RICE EST          *S*L******H*****ST**P****KY****L*.***R****M**FL****.****DLF**E***R***R******N**D******PSI*AG*F
AINTEGUMENTA    369 ED**NMTRQ*Y*AH***KSS**S**A*IY****R*HQH*R*Q****RVA*NKD.L***TFG*QE***E***V****FR*TN******ITR**VDR 462
MAIZE ORF       215 EE**SMTRQ**IAS***KSS**S**A*IY****R*HQH*R*Q***R*CGR*QG.PV**                                  271
TINY             33                          HPVY***RKR.NW***VSE*REPRK*SR.IW**TFPSPEM**R*H*V**LSIK*AS*IL**PDLAGSFPR 101
EREBP-2          96                          GRHY***R*R.PW**FA*E*RDPAKNGAR*W**T*E*AE***L******YRMR*SK*LL**PHRIGLN*P 164
```

Figure 5. Sequence comparison of AP2 domains from *Glossy15* and other AP2 domain genes. Shown are the AP2 domain amino-acid sequences from the deduced cDNAs of *Glossy15* (accession no. U41466), *Arabidopsis APETALA2* (ATU12546; Jofuku et al. 1994), a rice EST (D23002), *Arabidopsis AINTEGUMENTA* (U40256; Klucher et al. 1996), a maize ORF sequence (Z47554, ZMMHCF1), the tobacco ethylene-response element DNA-binding protein EREBP-2 (D38126; Ohme-Takagi and Shinshi 1995), and the *Arabidopsis TINY* gene (X94698, Wilson et al. 1996). The amino acids of the two AP2 domains (as defined in Jofuku et al. 1994) are indicated above the sequences, and the putative nuclear localization signal sequences are underlined. Asterisks represent amino acids identical to *Glossy15*; gaps included to optimize alignment are indicated by periods. The number at the beginning of each sequence is the number of amino-acid residues from the first methionine or the first amino acid of the reading frame. The single AP2 domain in EREBP-2 displayed similar degrees of identity (38%) when aligned to either of the two AP2 domains from *Glossy15*.

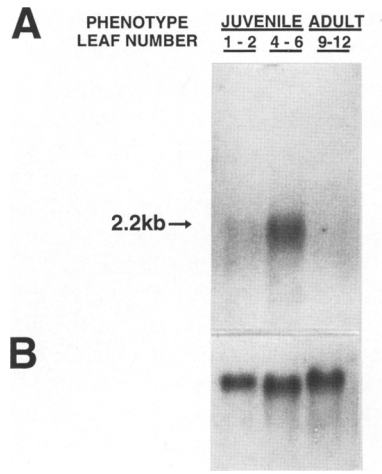

Figure 6. RNA gel-blot analysis of poly(A)$^+$ RNA isolated from maize leaves. Ten micrograms of poly(A)$^+$ RNA from W64A leaves 1 and 2 (juvenile phenotype), leaves 4–6 (juvenile phenotype), and leaves 9–12 (adult phenotype) were loaded per lane. Each RNA sample included only developing unexpanded leaf tissue within 10 cm of, but excluding, the shoot apex. (*A*) The blot was hybridized to the P700.G1 *Gl15* probe, which detected a 2.2-kb transcript from juvenile but not adult leaf mRNA. (*B*) The blot in *A* was stripped and rehybridized with a maize actin probe (Shah et al. 1983) as a control for RNA loading.

et al. 1994; Moose and Sisco 1994), *Gl15* may control the differentiation of a small proportion of basal epidermal cells within leaf 2 and thus account for the hybridization signal in leaves 1 and 2. It is also possible that there is another *Gl15*-related gene that controls the juvenile identity of the first two leaves, to which the P700.G1 probe may hybridize weakly. The *Gl15* transcript was not detected in adult leaf mRNA, consistent with our other observations that *Gl15* activity is correlated strictly with the juvenile epidermal phenotype beyond leaf 2.

Because of the low abundance of the *Gl15* transcript even in leaves 4–6, we exploited the sensitivity of reverse transcription–PCR (RT–PCR; Byrne et al. 1988) to further characterize *Gl15* expression during maize development. Figure 7 shows the results from one such RT–PCR experiment. Among leaves from normal plants, *Gl15* mRNA expression was observed as expected from leaves 4–6 but was not detected in either leaves 1 and 2 or adult leaves. These results, when compared with those in Figure 6, suggested that the hybridization signal obtained from leaves 1 and 2 in RNA gel-blot analysis may represent cross-hybridization with a putative *Gl15*-related gene that is expressed in early juvenile leaves. Very low levels of a *Gl15*-related RT–PCR product were detected in the plumule, or embryonic leaves, harvested from late-stage (32 days after pollination) developing embryos. Because the plumule contains the first 5–6 leaf primordia (Kiesselbach 1949; Abbe and Stein 1954), we could not determine whether the observed RT–PCR product represents only *Gl15* expression in leaves 3–6 or also includes the expression of a *Gl15*-related gene active in leaves 1 and 2.

Gl15 expression in mutant plants is also illustrated in Figure 7. No *Gl15* mRNA was detected in leaves 4–6 from the *gl15-H* deletion mutation, demonstrating that it is a null allele. *Gl15* transcripts were detected in mRNA isolated from leaves 14–16 of *Cg1* mutant plants, whereas in normal plants leaves of the same developmental age (leaves 9–12) exhibit an adult epidermal cell identity and do not express *Gl15* (Figs. 6,7). The ectopic expression of *Gl15* mRNA beyond leaf 8 in *Cg1* mutant plants is consistent with the genetic observations that *Gl15* is required for the near-constitutive expression of juvenile epidermal cell traits conditioned by *Cg1* and that it acts downstream of the *Cg1* mutation (Moose and Sisco 1994).

The RT–PCR analysis was not quantitative as performed. Thus differences in intensities of signals among samples in Figure 7 do not necessarily reflect the relative abundance of *Gl15* transcript present in the RNA sample. Successful reverse transcription was verified for each sample through the amplification of a 700-bp product from the maize *b-70* mRNA encoding the endoplasmic reticulum-localized molecular chaperone BiP (Fontes et al. 1991; see Materials and Methods). The results obtained from both RNA gel blots and RT–PCR are consistent with the presence of a distinct middle domain within the normal maize shoot (leaves 3–7 in the inbred W64A) and indicate that after leaf 2, a juvenile leaf identity requires the expression of the *Gl15* gene.

Discussion

Glossy15 is the first molecularly characterized gene that regulates the transition from juvenile to adult shoot de-

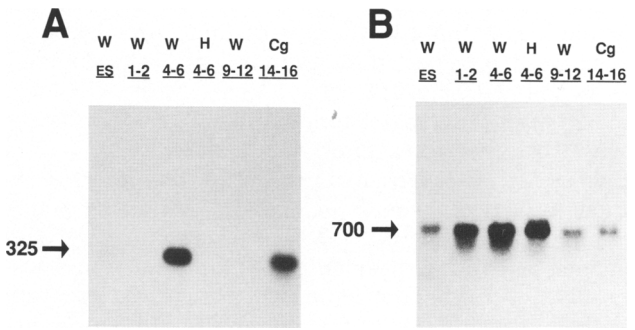

Figure 7. RT–PCR analysis of maize *Gl15* mRNA expression. First-strand cDNAs from W64A embryonic shoots, W64A leaves 1 and 2, W64A leaves 4–6, *gl15-H* leaves 4–6, W64A leaves 9–12, and *Cg1*/+ leaves 14–16 were amplified by PCR, resolved by agarose gel electrophoresis, blotted onto a nylon membrane, and hybridized to labeled probes as described in the Materials and Methods. (*A*) PCR amplification with the *Gl15*-specific primers shown in Fig. 4 and probed with the SP430.JS probe from *Gl15*. The arrow indicates the 325-bp product detected by the *Gl15* probe. (*B*) PCR amplification with primers from the *b-70* gene and probed with the entire *b-70* cDNA. The arrow indicates the 700-bp product detected by the *b-70* probe.

velopment in plants. The correlation of distinct RFLPs with the *gl15-m1* allele, its revertant derivatives, and the three *gl15-Ref* mutations served as evidence that the DNA flanking the *dSpm* insertion in *gl15-m1* represented a portion of the *Gl15* gene. Our characterization of *Gl15* mRNA expression, its predicted protein sequence, and comparisons to other related genes indicated that leaf epidermal cell identity in maize is determined by this AP2-domain transcription factor. This finding expands the known functions of *AP2*-related genes and suggests that similar genes in other plants may participate in the determination of vegetative as well as reproductive lateral organ identity.

The Gl15 protein has features of transcription factors

The Gl15 protein shares a number of similarities to known transcription factors. *Gl15* contains two repeated AP2 domains that both share identity with all 24 of the AP2 domain consensus residues as determined by Weigel (1995). The AP2 domain is a novel motif that is predicted to form an amphipathic α-helix and, in both *AP2* and *Gl15*, is flanked by a 10-amino-acid basic region proposed to function as a nuclear localization signal (Jofuku et al. 1994). Regions capable of forming an α-helical structure and having a high density of basic amino acids are consistent with a DNA-binding motif (Mitchell and Tjian 1989). The ability to form amphipathic α-helices suggests that the core region of the AP2 domain may mediate protein–protein interactions, possibly as a dimerization domain with other related proteins (Cohen and Parry 1994; Jofuku et al. 1994). The presence of two AP2 domains within *Gl15* may permit DNA binding by a single polypeptide, whereas genes with only a single AP2 domain, such as *EREBP-2* from tobacco, may require the formation of homo- or heterodimers for DNA binding activity. Additionally, *Gl15* has a serine-rich acidic region and a histidine/glutamine stretch (Fig. 4) that may serve as transcription activation domains (Mitchell and Tjian 1989).

A transcription factor function for *Gl15* is consistent with the pleiotropic effects of *gl15* mutations on a diverse set of epidermal traits and its cell-autonomous mode of action. Phenotypic analysis of *gl15-m1* plants demonstrated that *Gl15* both activates juvenile and suppresses adult leaf epidermal traits (Moose and Sisco 1994). Potential downstream targets of *Gl15* activity include genes involved in epicuticular wax biosynthesis, cell-wall metabolism, and cellular differentiation pathways. Interestingly, increased or ectopic expression of the *Arabidopsis TINY* gene, which encodes a protein with a single AP2 domain, conditions changes from an irregular to a rounded leaf epidermal cell shape (Wilson et al. 1996). *Gl15* activity also induces similar effects in the maize leaf epidermis. Nevertheless, the amino acid sequence of the TINY protein is significantly different from that of the double-AP2-domain proteins (Fig. 4), and *TINY* is clearly not the *Arabidopsis* ortholog of *Gl15*.

Gl15 *and the regulation of vegetative phase change in maize*

Shoot growth in maize can be divided into distinct juvenile and adult phases, which differ by the expression of a large set of traits, including the character of the leaf epidermis. Previous analysis of *gl15* mutations indicated the juvenile phase may be further subdivided into two separate juvenile regulatory programs, one operating in leaves 1 and 2, the other acting in leaves 3–7 (Evans et al. 1994; Moose and Sisco 1994). The *Gl15* mRNA expression pattern in normal plants and in the *gl15-H* null allele demonstrated the existence of three developmental domains within the shoot, each associated with distinct leaf epidermal cell identities. The basal 1–3 leaves are juvenile but regulated independently from *Gl15*; middle leaves have a juvenile identity that is promoted by *Gl15*; and upper leaves are adult. The replacement of juvenile with adult epidermal cell phenotypes in the middle leaves of *gl15* mutants suggests that *Gl15* suppresses adult epidermal cell differentiation and that the absence of *Gl15* activity conditions an adult epidermal cell fate. However, if *Gl15* does antagonize the activity of adult genes, then it must do so only in leaves 3–7, because upper leaves of *Cg1* plants possess both juvenile and adult epidermal cell types (Moose and Sisco 1994) but continue to express *Gl15* mRNA (Fig. 7).

The expression of *Gl15* itself appears to respond to the activity of factors that affect phase change globally, such as those defined by the *Cg1* and *Tp* mutations or possibly the gibberellin class of plant growth regulators (Evans and Poethig 1995). However, nothing is known at the molecular level about the upstream regulators of *Gl15* expression or their mechanism of action. Molecular and genetic studies in *Arabidopsis* have demonstrated that *AP2*, in conjunction with other factors, negatively regulates the expression of the MADS-box gene *AGAMOUS* (*AG*) in the outer two whorls of the *Arabidopsis* flower, resulting in the formation of perianth (sepals and petals) rather than sexual organs (Drews et al. 1991; Jofuku et al. 1994). Further work suggests that *AP2* cooperates with both *LEUNIG* (Liu and Meyerowitz 1995) and the MADS-box gene *APETALA1* (Irish and Sussex 1990) to mediate its effects on floral meristem and organ identity. Thus *Gl15* may interact with similar genes to regulate leaf identity in maize. Though most characterized MADS-box genes are expressed in developing flowers, vegetatively expressed MADS-box genes have been identified in a number of plant species, including maize (Pnueli et al. 1991; Mandel et al. 1994; Mena et al. 1995; Rounsley et al. 1995; Tandre et al. 1995). The isolation and characterization of loss-of-function mutations in these genes may reveal their role in regulating vegetative meristem or leaf identity.

AP2*-related genes regulate both vegetative and floral organ identity in plants*

A large number of genes have been identified that contain an AP2 domain (Weigel 1995). Among these genes,

only those of a relatively small subset possess two repeated AP2 domains. The presence of sequences related specifically to either *Gl15*, *AP2*, or *ANT* in both monocot and dicot species (Fig. 5 and data not shown) suggests that each of these three genes is descended from a common ancestral gene that underwent subsequent diversification during plant evolution. This diversification is reflected in the observed differences among *Gl15*, *AP2*, and *ANT* with respect to their mutant phenotypes, AP2-domain sequences, and expression patterns during plant development. The *AP2* and *ANT* genes are expressed in both vegetative and floral tissues, but exhibit a mutant phenotype only during reproductive development (Jofuku et al. 1994; Elliott et al. 1996; Klucher et al. 1996). This is in contrast to *Gl15*, whose mRNA expression pattern is correlated with the *gl15* mutant phenotype in juvenile vegetative leaves (Figs. 6 and 7). The possibility exists that *AP2* and *ANT* also function during vegetative development, but redundant gene activities prevent the observation of a vegetative phenotype in *ap2* or *ant* mutants. Genetic redundancy for *Gl15* activity may also account for the continued juvenile character of the first two leaves in *gl15* mutations (Evans et al. 1994; Moose and Sisco 1994).

If *Gl15*, *AP2*, and *ANT* are derived from a common ancestral gene, then to what extent have the functions of these genes been conserved? Mutations in *AP2* affect *Arabidopsis* floral meristem and floral organ identity, ovule formation, or the development of the seed coat, which is derived from the integuments in the ovule (Jofuku et al. 1994). *ANT* mutants affect mainly the initiation of lateral organs (the integuments) in the ovule, but also alter organ number and morphology in the outer three whorls of the flower (Elliott et al. 1996; Klucher et al. 1996). *Gl15* is required for a juvenile leaf epidermal cell identity during maize shoot development. Thus, each of these genes influence the formation of lateral organs (leaves, floral organs, or integuments) derived from developing meristems. Important differences exist, however, in where these *AP2*-related genes regulate this process. *AP2* regulates both floral meristem and floral organ identity, and therefore acts throughout reproductive development. *ANT* appears to have a general role in the initiation of lateral organs from both shoot and floral meristems. In contrast, the limited phenotypes and mRNA expression patterns of *Gl15* indicate that it functions specifically to control leaf epidermal cell identity.

The finding that *AP2*-related genes regulate lateral organ identity during both vegetative and reproductive development supports the hypothesis that leaves and floral organs are related structures derived from a common growth plan (Goethe 1790; Coen and Carpenter 1993). The presence of *Gl15*-related sequences in taxonomically diverse plant species suggests that similar genes may also regulate leaf identity in other heteroblastic plants. The continued characterization of *Gl15* and other maize genes that regulate its activity, such as *Cg1*, *Tp1*, and *Tp2*, should provide additional insights into the control of vegetative phase change and leaf identity in plants.

Materials and methods

Genetic stocks

The origin, identification, and genetic characterization of the *gl15-m1::dSpm* allele were described previously in Moose and Sisco (1994). The *gl15-H* and *gl15-S* stocks were obtained from the Maize Genetics Cooperation Stock Center (Urbana, IL), and the *gl15-L* stock from G.F. Sprague (United States Department of Agriculture/Agricultural Research Service, retired, Eugene, OR). Each of these mutations and a *Cg1/+* mutant obtained from R. Bertrand (Colorado College, Colorado Springs) had been backcrossed three times into the W64A inbred background. Revertant alleles (*Gl15'+*) derived from *gl15-m1::dSpm* were identified in plants exhibiting a heritable normal juvenile seedling phenotype and a *Wx/wx* genotype among F_1 progeny from the cross *gl15-m1*, *Wx*, *Spm* × *gl15-H*, *wx*. The kernel mutant *waxy* (*wx*), 10 cM from *gl15* (Coe 1993), was used as a genetic marker to control against wild-type (*Gl15*) pollen contamination. Candidate revertants were selfed, and progeny homozygous for the *Gl15'* alleles were selected. Crosses were performed by hand pollination either in a greenhouse or at a Clayton, NC, summer nursery.

DNA methods

The BX-1 *Spm* element probe was obtained from R. Schmidt (University of California at San Diego). Maize inbred W64A plants were grown from seed in a greenhouse and its genomic DNA was isolated by a CTAB procedure as described in Saghai-Maroof et al. (1984). DNA gel blots were prepared and hybridized to gel-purified, random primer radioactively labeled DNA fragments according to Sisco (1991), except 100 μg/ml herring sperm DNA and 5× Denhardt's solution (5× is 0.1% wt/vol Ficoll, 0.1% wt/vol BSA, 0.1% wt/vol polyvinylpyrollidone) were used instead of heparin. The hybridization temperature for the BX-1 probe was 75°C. All other hybridizations were carried out at 68°C. Filters were washed once for 20 min in 2× SSC, 0.15 M NaCl, 0.015 M sodium citrate, 0.1% SDS at 65°C, twice for 20 min in 1× SSC, 0.1% SDS at 65°C, and twice for 10 min in 0.1× SSC, 0.1% SDS at 65°C. Filters were autoradiographed at −80°C from 1 to 14 days.

Genomic cloning

DNA used in molecular cloning was prepared by a CTAB procedure (Saghai-Maroof et al. 1984). To obtain the 3.3-kb *Sst*I fragment representing *gl15-m1::dSpm*, a partial genomic DNA library was prepared from total genomic DNA of homozygous *gl15-m1* plants by size-selection and purification (GeneClean, BIO 101) of 3–4 kb *Sst*I fragments through an agarose gel. The purified fragments were ligated to *Sst*I–*Eco*RI linkers and cloned into dephosphorylated, *Eco*RI-digested λZap (Stratagene). Packaging into Gigapack Gold (Stratagene), plating on either SURE or XL1-Blue MRF host strains, and screening of this and subsequent libraries was carried out according to the manufacturer's protocols. Three BX-1 hybridizing clones were obtained from ~1×10^6 plaques and converted to pBluescript phagemids by the recommended in vivo excision protocol (Stratagene). One plasmid possessed a 3.3-kb *Sst*I insert with an internal 2.1-kb *dSpm* element flanked by maize genomic sequences. A 1.1-kb *Hin*dIII–*Sst*I fragment (HS1100.43, Fig. 2) was isolated from this clone and used in subsequent analyses.

Genomic clones for the *Gl15-W64A* allele were obtained from a total W64A genomic DNA library. Total genomic DNA was partially digested with *Sau*3AI, and partially filled in with dATP and dGTP. Fragments from 9 kb to 25 kb were selected and

purified from agarose gels (Weil and Bureau 1994). Prepared fragments were subsequently ligated to the λFixII vector (Stratagene), packaged, and ~1×10^6 recombinant clones screened with the HS1100.43 probe. Eleven hybridizing clones were isolated, nine of which represented overlapping clones from the *Gl15* locus. The 6.0-kb *Eco*RI fragment from one of these clones (G6) that hybridized to HS1100.43 was subcloned into pBluescript KS+ (Stratagene).

cDNA cloning and sequence analysis

Total RNAs from W64A shoots harvested at 3, 6, 9, and 13 days after kernel sowing (DAS) were isolated using a protocol modified from Chirgwin (1979; M. Redinbaugh, pers. comm.). The 9 DAS and 12 DAS samples included only young developing leaves 4–6 and the shoot apex. Equal amounts of RNA (by weight) from each time point were pooled together. This pooled sample was intended to represent all possible developmental stages of both early juvenile and late juvenile leaves. Poly(A)$^+$ RNA was selected by passage through oligo(dT) cellulose columns (Clonetech) and ~5 μg of juvenile shoot mRNA was used to construct a cDNA library with the ZAP-cDNA Synthesis Kit (Stratagene) according to the manufacturer's instructions. Six *Gl15* cDNA clones were identified by screening 5×10^5 primary recombinant plaques with the P700.G1 probe. These were converted to pBluescript phagemids by in vivo excision and determined to carry insert lengths from 1.8 kb to 2.3 kb. Portions of each of these clones were subcloned into pBluescript vectors.

Subclones from the *gl15-m1* genomic clone, the 6.0-kb *Eco*RI *Gl15*-W64A genomic clone, and W64A *Gl15* cDNA clones were prepared by use of standard techniques. Both strands from these clones were sequenced using standard Sequenase (U. S. Biochemical) protocols or through the Applied Biosystems (Foster City, CA) sequencers at Iowa State University, Ames. DNA sequence analysis was performed using the programs (version 8) of the Wisconsin Genetics Computer Group (Madison, WI), and data-base searches were conducted using the BLAST network service at the National Center for Biotechnology Information at the National Library of Medicine (Bethesda, MD).

RNA analysis

Total RNAs were isolated (as above) from greenhouse-grown W64A plants at the following developmental stages: leaves 1 and 2 at 6 days after sowing (DAS), leaves 4–6 at 12 DAS, and leaves 9–12 at 33 DAS. Each sample included both developing and fully differentiated leaf tissue. Poly(A)$^+$ RNA was selected by passage through oligo(dT) spin columns (Clontech). mRNAs (10 μg) were electrophoresed through a 1.3% formaldehyde gel and transferred to GeneScreen+ membrane (DuPont) in 0.05 M NaOH for 6 hr. Hybridizations and washes were conducted as for high-stringency genomic DNA blots with the membrane sandwiched between two pieces of 3MM Whatman filter paper (Jones and Jones 1992).

Total RNAs from the above W64A leaf samples, developing W64A embryonic axes dissected from kernels at 35 days after pollination, leaves 4–6 of homozygous *gl15-H* plants at 12 DAS, and leaves 14–16 of heterozygous *Cg1*/+ plants at 33 DAS were used to amplify the *Gl15* transcript by RT–PCR. The *gl15-H* and *Cg1* mutant alleles had been backcrossed three times into a W64A inbred background. First-strand cDNA was synthesized using 5 μg of total RNA at 48°C for 1 hr using oligo(dT) ($n = 15$) and SuperScriptII modified reverse transcriptase (Bethesda Research Laboratories) as described by the manufacturer. PCR was performed on 2 μl of the first-strand cDNA reaction in a final volume of 100 μl, using either the *Gl15*-specific primers shown in Figure 3 or the following primers from the maize *b-70* gene (GenBank accession no. M59449): 5′-CCTGCGACTTCTTGGTCGGGAT-3′ and 5′-CTTGTCTTTGACCTTGGTGGTG-3′. The reactions were initially denatured at 97°C for 3 min, followed by 3 cycles of 97°C for 1 min, 55°C for 1 min, and 72°C for 1 min. Thirty-five additional cycles were conducted at 94°C for 1 min, 55°C for 1 min, and 72°C for 90 sec. Thirty microliters from each PCR was electrophoresed through agarose gels, blotted to nylon membrane, and hybridized to either *Gl15* or *b-70* probes as described above.

Acknowledgments

We thank Russell Wrobel, Ralph Dewey, John Goode, Lynn Senior, and William Thompson for helpful advice regarding DNA gel blots, library construction, and DNA sequencing. We are grateful to Christian Kermorvant and Nichole Sprouse for their assistance with gel blots and DNA sequencing, William G. Brown, Jr., for excellent care of greenhouse and nursery plants, and Wilma Hu for the photography of figures and help with scanning electron microscopy. We thank Rebecca Boston and R. Wrobel for the maize *b-70* gene primers, Robert Schmidt for the BX-1 *Spm* probe, and the laboratories of Sarah Hake, Robert Fischer, and David Smyth for communicating unpublished data. Michael Lee, Michael Purugganan, Ralph Dewey, and Ross Whetten supplied genomic DNAs used in the cross-species analysis. This work was supported by USDA/ARS CWU 6645-21000-006-00D and USDA-National Research Initiative Competitive Grant 93-37304-8916 to P.H.S. S.P.M. was supported by a Plant Molecular Biology Fellowship from the North Carolina Biotechnology Center, Research Triangle Park.

The publication costs of this article were defrayed in part by payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact.

Note

The sequence data described in this paper for *Gl15* cDNA have been submitted to GenBank under accession no. U41466.

References

Abbe, E.C. and O.L. Stein. 1954. The growth of the shoot apex in maize: Embryogeny. *Am. J. Bot.* **41:** 285–293.

Allsopp, A. 1967. Heteroblastic development in plants. In *Advances in morphogenesis* (ed. M. Abercrombie and J. Brachet), pp. 127–171. Academic Press, New York, NY.

Byrne, B.C., J. Li, J. Sninsky, and B.J. Poiesz. 1988. Detection of HIV-1 RNA sequences by in vitro DNA amplification. *Nucleic Acids Res.* **16:** 4165.

Chirgwin, J.J., A.E. Przbyla, R.J. MacDonald, and W.J. Rutter. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* **18:** 5294–5299.

Coe, E.H., Jr. 1993. Gene list and working maps. *Maize Genet. Coop. News Lett.* **67:** 165.

Coen, E.S. and R. Carpenter. 1993. The metamorphosis of flowers. *Plant Cell* **5:** 1175–1181.

Cohen, C. and D.A.D. Parry. 1994. α-helical coiled-coils: More facts and better predictions. *Science* **263:** 488–489.

Cone, K.C., F.A. Burr, and B. Burr. 1986. Molecular analysis of the maize anthocyanin regulatory locus *C1*. *Proc. Natl. Acad. Sci.* **83:** 9631–9635.

Drews, G.N., J.L. Bowman, and E.M. Meyerowitz. 1991. Nega-

tive regulation of the *Arabidopsis* homeotic gene *AGAMOUS* by the *APETALA2* product. *Cell* **65:** 991–1002.

Elliott, R.E., A.S. Betzner, E. Huttner, M.P. Oakes, W.Q.J. Tucker, D. Gerentes, P. Perez, and D.R. Smyth. 1996. *AINTEGUMENTA*, an *APETALA2*-like gene of Arabidopsis with pleiotropic roles in ovule development and floral organ growth. *Plant Cell* **8:** 155–168.

Evans, M.M.S. and R.S. Poethig. 1995. Gibberellins promote vegetative phase change and reproductive maturity in maize. *Plant Physiol.* **108:** 475–487.

Evans, M.M.S., H.J. Passas, and R.S. Poethig. 1994. Heterochronic effects of *glossy15* mutations on epidermal cell identity in maize. *Development* **120:** 1971–1981.

Fontes, E.B.P., B.B. Shank, R.L. Wrobel, S.P. Moose, G.R. O'Brian, E.T. Wurtzel, and R.S. Boston. 1991. Characterization of an immunoglobulin binding protein homolog in the maize *floury-2* endosperm mutant. *Plant Cell* **3:** 483–496.

Galinat, W.C. 1966. The corn grass and teopod loci involve phase change. *Maize Genet. Coop. News Lett.* **40:** 102–103.

Goebel, K. 1900. *Organography in plants*. Oxford University Press, Oxford, UK.

Goethe, J.W. 1790. Versuch die Metamorphose der Pflanzen zu erklären, transl. A. Arber (1946). *Chron. Bot.* **10:** 63–115.

Irish, V.F. and I.M. Sussex. 1990. Function of the *APETALA1* gene during *Arabidopsis* floral development. *Plant Cell* **2:** 741–753.

Jofuku, K.D., B.G.W. den Boer, M. Van Montagu, and J.K. Okamuro. 1994. Control of *Arabidopsis* flower and seed development by the homeotic gene *APETALA2*. *Plant Cell* **6:** 1211–1225.

Jones, R.W. and M.J. Jones. 1992. Simplified filter paper sandwich blot provides rapid, background-free Northern blots. *BioTechniques* **12:** 685–687.

Kiesselbach, T.A. 1949. *The structure and reproduction of corn*. University of Nebraska Press, Lincoln, NE.

Klucher, K., H. Chow, L. Reiser, and R.L. Fischer. 1996. The *AINTEGUMENTA* gene of *Arabidopsis* required for ovule and female gametophyte development is related to the floral homeotic gene *APETALA2*. *Plant Cell* **8:** 137–153.

Lawson, E.J.R. and R.S. Poethig. 1995. Shoot development in plants: Time for a change. *Trends Genet.* **11:** 263–268.

Liu, Z. and E.M. Meyerowitz. 1995. *LEUNIG* regulates *AGAMOUS* expression in *Arabidopsis* flowers. *Development* **121:** 975–991.

Mandel, T., I. Lutziger, and C. Kuhlemeier. 1994. A ubiquitously expressed MADS-box gene from *Nicotiana tabacum*. *Plant Mol. Biol.* **25:** 319–321.

Mena, M., M.A. Mandel, D.R. Lerner, M.F. Yanofsky, and R.J. Schmidt. 1995. A characterization of the MADS-box gene family in maize. *Plant J.* **8:** 845–854.

Mitchell, P.J. and R. Tjian. 1989. Transcriptional regulation in mammalian cells by sequence-specific DNA binding proteins. *Science* **245:** 371–378.

Moose, S.P. and P.H. Sisco. 1994. *Glossy15* controls the epidermal juvenile-to-adult phase transition in maize. *Plant Cell* **6:** 1343–1355.

Nash, J. and V. Walbot. 1992. Bronze-2 gene expression and intron splicing patterns in cells and tissues of *Zea mays* L. *Plant Physiol.* **100:** 464–471.

Ohme-Takagi, M. and H. Shinshi. 1995. Ethylene-inducible DNA binding proteins that interact with an ethylene-responsive element. *Plant Cell* **7:** 173–182.

Pnueli, L., M. Abu-Abeid, D. Zamir, W. Nacken, Zs. Schwarz-Sommer, and E. Lifschitz. 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.

Poethig, R.S. 1988. Heterochronic mutations affecting shoot development in maize. *Genetics* **119:** 959–973.

———. 1990. Phase change and the regulation of shoot morphogenesis in plants. *Science* **250:** 923–930.

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

Saghai-Maroof, M., K. Soliman, R. Jorgenson, and R. Allard. 1984. Ribosomal DNA spacer length polymorphism in barley: Mendelian inheritance, chromosomal location, and population dynamics. *Proc. Natl. Acad. Sci.* **81:** 8014–8018.

Shah, D.M., R.C. Hightower, and R.B. Meagher. 1983. Genes encoding actin in higher plants: Intron positions are highly conserved but the coding sequences are not. *J. Mol. Appl. Genet.* **2:** 11–26.

Sisco, P.H. 1991. Duplications complicate genetic mapping of *Rf4*, a restorer gene for cms-C cytoplasmic male sterility in corn. *Crop Sci.* **31:** 1263–1266.

Smith, L.G. and S. Hake. 1992. The initiation and determination of leaves. *Plant Cell* **4:** 1017–1027.

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

Varagona, M. J., R.J. Schmidt, and N.V. Raikhel. 1992. Nuclear localization signal(s) required for nuclear targeting of the maize regulatory protein Opaque-2. *Plant Cell* **4:** 1213–1227.

Weigel, D. 1995. The APETALA2 domain is related to a novel type of DNA binding domain. *Plant Cell* **7:** 388–389.

Weil, C. F. and T.F. Bureau. 1994. Construction of a genomic library in lambda phage. In *The maize handbook* (ed. M. Freeling and V. Walbot), pp. 595–598. Springer-Verlag, New York, NY.

Wilson, K., D. Long, J. Swinburne, and G. Coupland. 1996. A *Dissociation* insertion causes a semidominant mutation that increases expression of *TINY*, an *Arabidopsis* gene related to *APETALA2*. *Plant Cell* **8:** 659–671.

Glossy15, an APETALA2-like gene from maize that regulates leaf epidermal cell identity.

S P Moose and P H Sisco

Genes Dev. 1996, **10:**
Access the most recent version at doi:10.1101/gad.10.23.3018