The Plant Cell, Vol. 6, 1343–1355, October 1994 © 1994 American Society of Plant Physiologists

RESEARCH ARTICLE

Glossy15 Controls the Epidermal Juvenile-to-Adult Phase Transition in Maize

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Loss-of-function mutations at the maize *Glossy15* (*Gl15*) locus alter the normal transition from juvenile-to-adult growth by conditioning the abbreviated expression of juvenile epidermal cell traits and the coordinate precocious expression of adult epidermal cell features. These include epicuticular wax composition, cell wall characteristics, and the presence or absence of differentiated epidermal cell types (e.g., epidermal macrohairs and bulliform cells). A transposon-induced mutable allele of *Glossy15* (*gl15-m1*) was isolated and employed in both phenotypic and genetic analyses to characterize the role of *Gl15* in the maize juvenile-to-adult phase transition. Comparisons between *Gl15*-active and *Gl15*-inactive somatic sectors in the leaves of variegated plants demonstrated that the *Gl15* gene product acts in a cell-autonomous manner to direct juvenile epidermal differentiation but does not affect factors that regulate the overall process of phase change. Examination of the *gl15-m1* phenotype in the *Corngrass1*, *Teopod1*, and *Teopod2* mutant backgrounds showed that the prolonged expression of juvenile epidermal traits associated with these mutations also required *Gl15* activity. These results support a model whereby the cell-autonomous *Gl15* gene product responds to a juvenility program that operates throughout the vegetative shoot to condition the juvenile differentiation of maize leaf epidermal cells.

INTRODUCTION

Many plants, including maize, exhibit distinct juvenile and adult phases of vegetative development (reviewed by Poethig, 1990). Table 1 shows that the juvenile and adult phases differ in their leaf shape, reproductive capacity, and expression of a set of morphological and biochemical leaf epidermal traits. Most maize genotypes express juvenile traits exclusively for the first five to six nodes. Beginning with nodes 6 or 7, juvenile traits gradually disappear and are coordinately replaced by adult features. From node 8 to the terminal tassel, vegetative tissues are exclusively adult.

Previous studies have demonstrated that the maize juvenile-to-adult phase transition is under genetic control, as evidenced by the phenotypes of four independent mutations that prolong the transition from juvenile-to-adult vegetative growth. The *Corngrass1* (*Cg1*), *Teopod1* (*Tp1*), *Teopod2* (*Tp2*), and *Hairy sheath-frayed1-O* (*Hsf1-O*) genes represent semidominant, gain-of-function mutations that extend the expression of the juvenile developmental program such that juvenile traits are observed along with the normal adult features, giving rise to nodes with intermediate phenotypes (Galinat, 1966a; Poethig, 1988a; Bertrand-Garcia and Freeling, 1991). These mutations affect the entire set of traits associated with the vegetative phase transition (Table 1). Additional work has shown that the expression of the *Tp1* and *Tp2* mutations is non-cell autonomous (Poethig, 1988b; Dudley and Poethig, 1993).

Mutant alleles of the *Glossy15* (*Gl15*) locus also affect phase change (Evans and Poethig, 1991; Evans et al., 1994). In addition to the previously reported effects of recessive *gl15* mutations on epicuticular waxes (Avato, 1987; Coe et al., 1988), these researchers showed that *gl15* conditions the replacement of juvenile with adult characteristics in the leaf epidermis beginning with nodes 2 or 3. Unlike the *Cg1*, *Tp*, and *Hsf* mutations, *gl15* appeared to affect only epidermal phase transition traits. Analysis of *Tp1 gl15* and *Tp2 gl15* double mutants also showed that *gl15* acts downstream of *Tp1* and *Tp2* (Evans et al., 1994).

In this article, we report the results of phenotypic and genetic analyses of a *defective Suppressor-mutator/Inhibitor* (*dSpm/I*)–induced mutable allele of the *Gl15* locus (*gl15-m1*). The *gl15-m1* mutation was allelic to three spontaneously occurring *gl15* mutations (Sprague, 1933; Hayes, 1939; Evans and Poethig, 1991). We demonstrate that *Gl15* acts in a cell-autonomous manner and is required for the expression of juvenile epidermal traits. Microscopic examination of the *gl15-m1* phenotype showed that *Gl15* coordinately activates the expression of cell-specific juvenile epidermal traits (waxes and cell wall characteristics) and suppresses the differentiation of adult epidermal cell types (e.g., bulliform cells and epidermal hairs). In addition, *gl15-m1*

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Table 1. Traits That Distinguish the Maize Juvenile and Adult Vegetative Phases[a]

Traits[b]	Juvenile	Adult
Adventitious roots	Present	Absent
Lateral buds	Tiller-like	Ears or absent
Leaf shape	Narrow	Broad
Internode distance	Compressed	Extended
Anthracnose resistance	Poor	Good
Epicuticular waxes (E)	Dull blue, fatty alcohols	Reduced levels, wax esters
Bulliform cells (E)	Absent	Present
Epidermal hairs (E)	Absent	Present
Cell wall invagination (E)	Moderate	Extreme
Toluidine blue O staining (E)	Violet	Aqua
Cell shape (cross-section) (E)	Rounded	Rectangular
Cuticle thickness (E)	<1 mm	>1 mm

[a] Data are summarized from Bianchi and Avato (1984), Lyons and Nicholson (1989), Poethig (1990), and Sylvester et al. (1990).
[b] E indicates epidermis-specific traits.

appeared to affect only epidermal traits. No differences were observed in the expression of nonepidermal features associated with phase change; these include lateral vegetative structures, leaf shape, and reproductive development. Analysis of the *gl15-m1* phenotype in the wild type and *Cg1*, *Tp1*, *Tp2*, and *Hsf1-O* mutant backgrounds showed that *Gl15* acts downstream of *Cg1*, *Tp1*, and *Tp2* but does not interact with *Hsf1-O*. In each of these genotypes, adult epidermal traits appeared at node 3, and revertant juvenile epidermal sectors were confined to those nodes where all juvenile phenotypes were expressed. *Gl15* mutations therefore uncouple epidermal differentiation from the overall process of phase change in the vegetative shoot.

We have demonstrated that the recessive *gl15-m1* mutation represents a loss-of-function and cell-autonomous defect in the signaling pathway regulating the maize juvenile-to-adult phase transition. Our results support a model whereby the cell-autonomous *Gl15* gene product responds to a regulatory factor(s) that operates throughout the shoot to condition the juvenile differentiation of maize leaf epidermal cells beginning with node 3.

RESULTS

Identification of *gl15-m1*::*dSpm/I*

In 1990, plants showing a variegated *glossy* mutant phenotype were discovered in a single F_2 family from a random transposon mutagenesis experiment where transpositions of *Activator* (*Ac*) from the *P-vv*::*Ac* allele were recovered by an excision assay (as detailed in Dellaporta and Moreno, 1994). This *Ac* mutagenesis stock was also later found to contain autonomous *Spm/Enhancer* (*En*) at unknown chromosomal locations (see Methods). Figure 1A shows that *glossy* mutant sectors appeared beginning with the third leaf. The delayed glossy phenotype suggested that the transposon-induced mutation was in the *gl15* gene. Standard tests for allelism using stocks of all the genetically mapped *glossy* mutants confirmed that the mutation was an allele of *gl15* on the long arm of chromosome 9. This allele was designated *gl15-m1*. Additional evidence that the mutable gene was *gl15* came from crosses of *gl15-m1* plants to a maize B-A translocation stock (Beckett, 1978), deficient for most of the long arm of chromosome 9. The resulting hemizygous F_1 progeny exhibited a *gl15-m1* phenotype identical to *gl15-m1* homozygotes. Restriction fragment length polymorphism loci known to flank the *gl15* locus (Howell et al., 1991) were also found to be linked to *gl15-m1* (data not shown).

Results from three independent genetic tests demonstrated a *dSpm/I* insertion at *gl15-m1*. First, 24 crosses of variegated *gl15-m1* plants to a stock carrying the *c-m(r)::I* reporter allele (a gift from P.A. Peterson, Iowa State University, Ames) yielded purple-variegated F_1 kernels, demonstrating the presence of autonomous *En/Spm* elements. This correlation was not observed from similar crosses to a stock carrying the *r-sc:m3*::*Dissociation* (*Ds*) reporter allele (Kermicle, 1984) for *Ac* activity. Second, testcrosses of homozygous *gl15-m1* plants to a *gl15* stock that lacked *Spm/En* (P.H. Sisco and S.P. Moose, unpublished data) segregated for *gl15*-variegated and *gl15*-stable phenotypes. Thus, mutability at *gl15* did not always cosegregate with *gl15-m1*, suggesting a *dSpm/I* rather than an autonomous *Spm/En* insertion. Finally, *gl15*-stable plants that were recovered from a stock homozygous for *gl15-m1* but that lacked active *Spm/En* were crossed with stocks of the inbred 4Co63 carrying active *En/Spm* elements. Peterson (1990) and Nelson (1991) reported that some but not all stocks of 4Co63 contain *En/Spm*. Our stocks did carry the *En/Spm* element as evidenced by testcrosses that transactivated the *c-m(r)::I* allele. Some of the resulting F_2 progeny showed a *gl15*-variegated phenotype, indicating the reactivation of a *dSpm/I* insertion at *gl15-m1* by *Spm/En*. The complete designation of the allele is thus *gl15-m1*::*dSpm/I* according to the rules for maize genetic nomenclature proposed by Beavis et al. (1993).

gl15-m1 Affects Epidermal Phase Transition Traits in a Cell-Autonomous Manner

The most obvious phenotypic marker that distinguishes juvenile from adult vegetative development is the production of epicuticular waxes. Most maize inbred backgrounds, such as inbred W64A, have a homogeneous juvenile wax phenotype for the first five nodes. Nodes 6 and 7 are transition nodes where the expression of juvenile waxes is disactivated and replaced with adult waxes (Figure 1F). This transition phenotype is observed earlier, at nodes 3 or 4, in *gl15-m1* plants. Figure 1 shows that both leaf blades and sheaths of *gl15-m1*

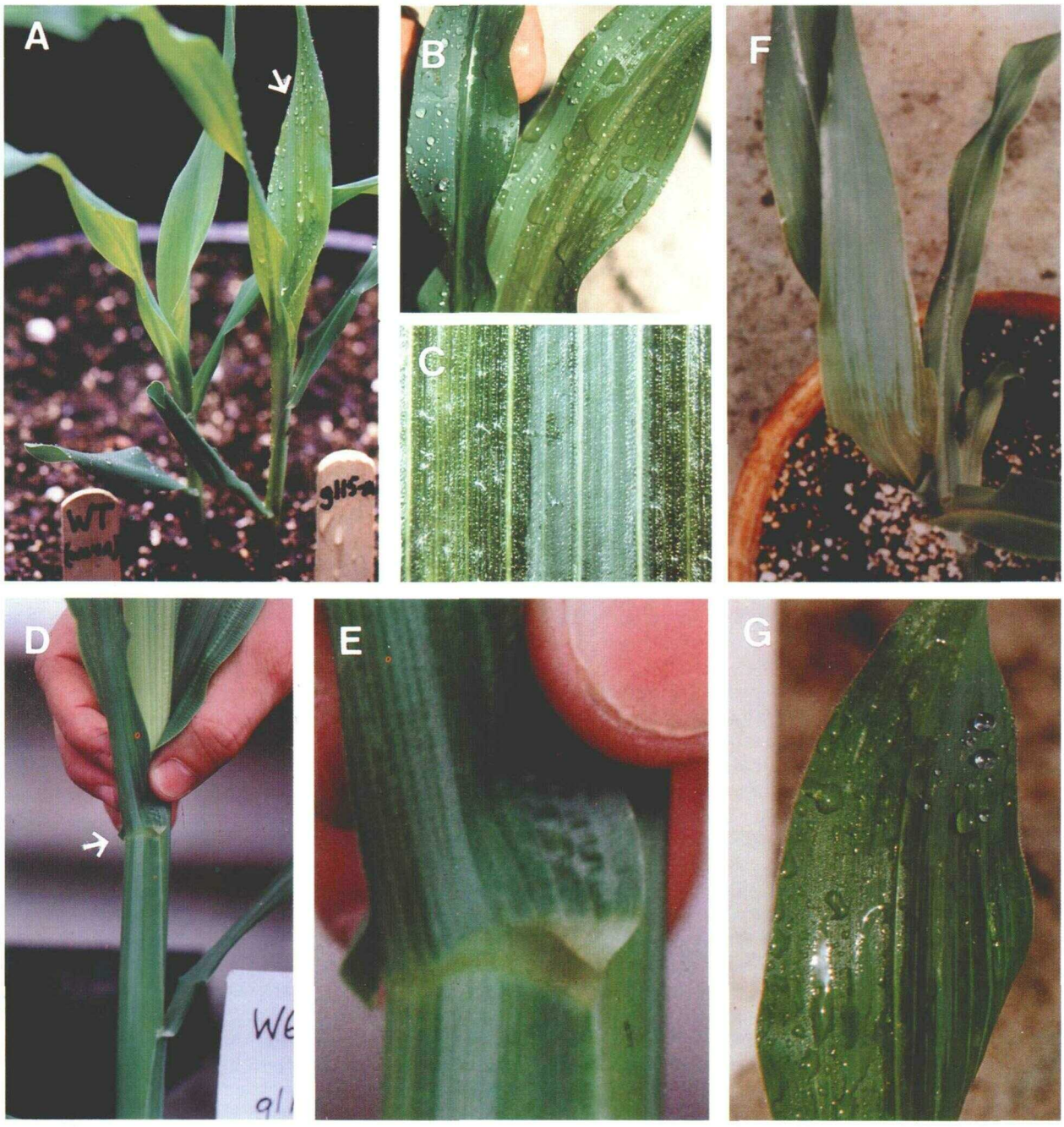

Figure 1. The *gl15-m1* Epicuticular Wax and Epidermal Macrohair Phenotypes.

(A) Wild-type (W64A inbred, left) and *gl15-m1* (right) seedlings misted with water. The arrow points to a somatic revertant wax sector present on leaf 4 of the *gl15-m1* plant.

(B) Lower surface from leaf 4 (held in hand) and upper surface from leaf 5 of *gl15-m1* seedling leaves misted with water. The *glossy* mutant sectors producing adult epicuticular waxes appear shiny green and fail to repel water; sectors of normal juvenile epicuticular waxes appear blue and cause water to bead up.

(C) Close-up view of the upper surface from leaf 4 of a *gl15-m1* seedling showing juvenile (bluish waxes, no macrohairs) and adult (transparent waxes, epidermal macrohairs) epidermal sectors.

(D) A revertant sector of juvenile waxes caused by a transposition that occurred in the lower leaf blade epidermis and extended through the ligule into the sheath. The arrow points to the ligular region shown as a close-up in **(E)**.

(E) Close-up of the sector shown in **(D)** featuring the blade–ligule–sheath junction.

(F) Lower surface of leaf 6 from the inbred W64A showing the normal transition from juvenile-to-adult epidermal wax.

(G) A revertant juvenile wax sector from the tip of leaf 6 of a *gl15-m1* plant at the position corresponding to the normal juvenile-to-adult phase transition boundary shown in **(F)**.

plants displayed clonal, somatic sectors of apparently normal juvenile waxes on an otherwise glossy leaf. Large juvenile wax sectors were coincident within both the abaxial and adaxial epidermis, whereas smaller sectors were generally found on either the abaxial or adaxial surface with no corresponding sector on the other side. Sectors that resulted from a transposition event in the leaf blade often continued into the sheath (Figure 1D). However, as in normal plants, juvenile epicuticular wax production was interrupted by the differentiated ligule at the leaf blade–sheath junctions (Figure 1E). Figure 1G shows a revertant juvenile wax sector from leaf 6 at the position corresponding to the normal juvenile-to-adult phase transition boundary (Figure 1F). The sector dissipated in a diffuse fashion among later cell divisions comprising the middle of the leaf. The gradual loss of revertant juvenile wax sectors was also observed in the lower leaf sheaths (Figure 1D). In addition, the size and frequency of juvenile wax sectors decreased with each successive node until the eighth node, where they were no longer observed. Similar to normal plants, *gl15-m1* plants had homogeneous adult waxes from node 8 to the tassel. Thus, the positional effects on the expression of revertant juvenile wax sectors indicated that factors defining the phase transition boundary in a normal plant were not affected by the *gl15-m1* mutation.

Evans et al. (1994) have recently reported that in addition to its effects on epicuticular waxes, *gl15* mutations also accelerate the expression of leaf hairs, aqua toluidine blue O staining, and angular cell shapes. Each of these epidermal traits is specific to the adult phase of vegetative development. We investigated the expression patterns of the above traits in *gl15-m1* plants. Figure 1C shows that leaf macrohairs were present in glossy mutant sectors on leaves as early as node 3, but not within revertant sectors producing juvenile epicuticular waxes. Other epidermal features were examined by preparing leaf blade epidermal peels and staining with toluidine blue O.

Representative peels from nodes 2 through 8 of W64A inbred and *gl15-m1* plants are shown in Figure 2. The juvenile upper epidermis (Figure 2A) appeared as a relatively undifferentiated tissue of similarly shaped elongated cells that stained violet with toluidine blue O. Stomatal complexes regularly interrupted the longitudinal files of epidermal cells, with subsidiary cells staining violet and guard cells aqua. Veins stained an intense blue. The juvenile epidermal cell walls possessed a wavy shape along their longitudinal axes while their lateral axes were relatively linear. No significant differences were observed between peels obtained from the upper or lower epidermis, except for an increased frequency of stomatal complexes in the lower epidermal cell layer (data not shown). Adult upper epidermal tissue (Figure 2B) was organized into a regular array of different cell types. Purple-staining files of square-shaped bulliform cells, which were three to five cells wide, appeared at fairly regular intervals and were immediately flanked by prickle hairs. Macrohairs were found within the files of bulliform cells. Alternating with the bulliform cells were 15 to 20 files of aqua-staining elongated cells with highly invaginated cell walls. Generally, blue veins and two to four files of stomatal complexes were present within the aqua-staining cell type. The adult phenotype of the lower epidermis was relatively undifferentiated, and only the aqua, highly invaginated cell type was present along with veins and a relatively higher frequency of stomatal complexes (data not shown). The distribution of epidermal cell phenotypes present at the normal juvenile-to-adult phase transition boundary is shown in Figures 2C and 2D. Individual cells expressed either juvenile or adult traits independently of their neighboring cells within either the same or adjacent longitudinal cell files. In addition, the transition from a juvenile to an adult epidermal cell fate occurred with a defined polarity in both a longitudinal tip-to-base (Figure 2C) and lateral margin-to-midrib (Figure 2D) direction. These observations indicated that phase change in the epidermis is cell autonomous but is also regulated by supracellular mechanisms.

The juvenile-to-adult phase transition in variegated leaves from *gl15-m1* plants is shown in Figures 2E and 2F. The stained peel from the *gl15-m1* upper epidermis (Figure 2F) possessed variably sized sectors of violet-staining juvenile cells that precisely coincided with the visible sectors of juvenile epicuticular waxes observed prior to removal and staining of the epidermis (Figure 2E). In addition to the large somatic revertant sector on the left side of the section, small sectors consisting of individual cells or groups of cells were also present. The small sectors were presumably a result of somatic transposition events that happened late in development, whereas the large sectors represent early transpositions. Single-cell sectors stained violet and had invaginated cell walls, conforming to the shape of the cell walls of their adult cell neighbors, whereas juvenile cells in the interior regions of larger sectors had the less invaginated, wavy cell wall shapes typical of juvenile cells. Small, random juvenile cell sectors and larger clonal sectors were unique to *gl15-m1* plants. Because sector boundaries, as visualized by toluidine blue O staining, were always coincident with and defined by cell walls, we concluded that *Gl15* acts in a cell-autonomous fashion. Consistent with this result is the previous finding that epicuticular wax production is cell autonomous (Freeling and Lane, 1994).

The cell-autonomous nature of revertant sectors was also evident in cross-sections of *gl15-m1* leaf tissues. Figure 3 shows a transverse view of leaf 4 from a *gl15-m1* plant. In the lower epidermis, a revertant sector of violet-staining, rounded juvenile cells can be seen adjacent to mutant tissue containing relatively rectangular aqua-staining adult cells. In the upper epidermis, an adult, nonrevertant sector can be seen consisting of a file of six purple-staining taller bulliform cells, a flanking microhair, and aqua-staining cells to the left of the bulliform cells. Differences in cell shape between violet-staining juvenile cells and aqua-staining adult cells were not as clear in the upper as in the lower epidermis. Cuticle thickness, another feature reported to distinguish the juvenile and adult epidermis (Poethig, 1990), showed no significant differences in these sections. Evans et al. (1994) have also found that changes in cuticle thickness were independent of the effects of *gl15* mutations.

The *gl15-m1* mutation in both the W64A and W23 inbred backgrounds had no apparent effect on the nonepidermal

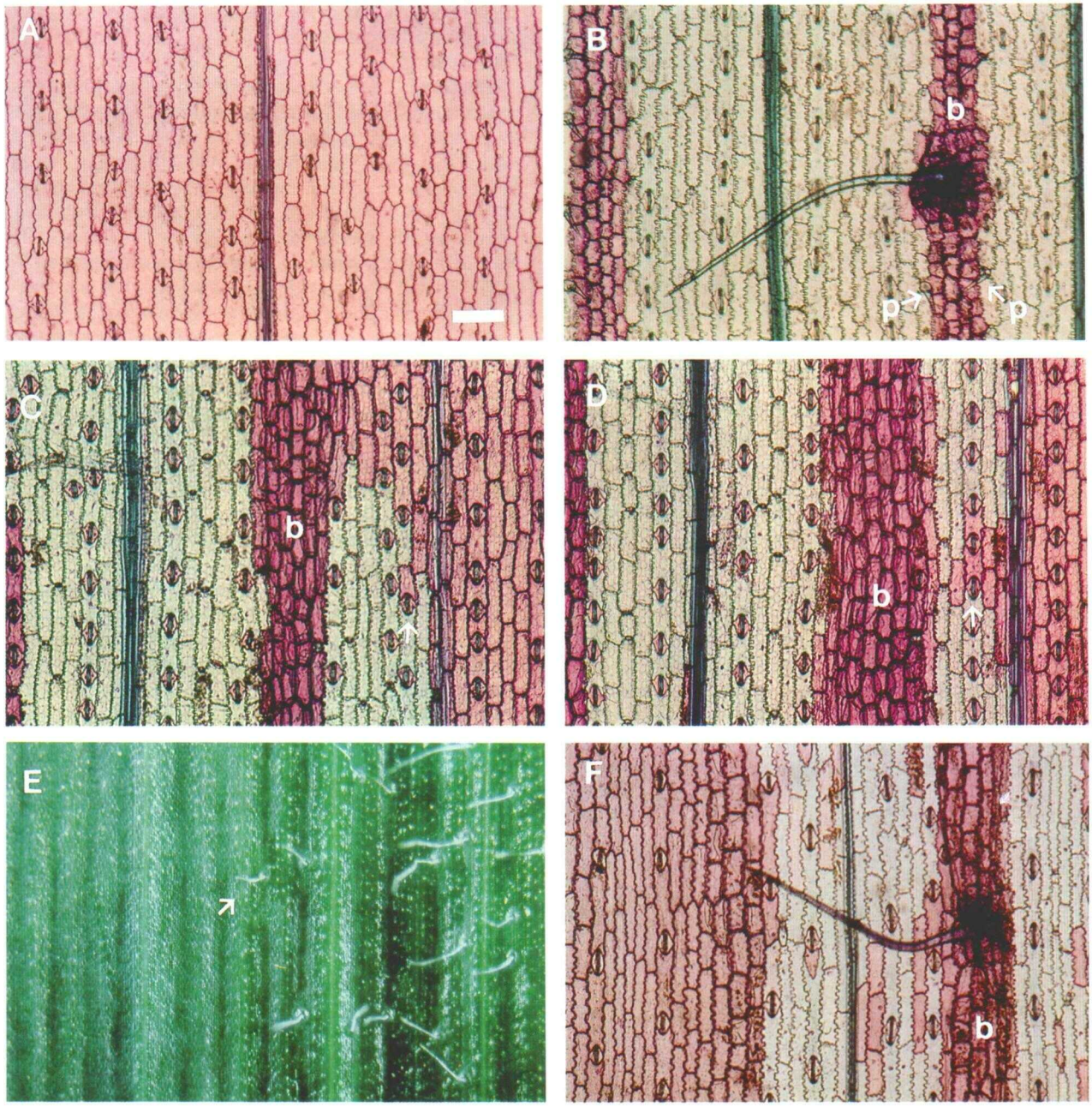

Figure 2. Toluidine Blue O Staining of Upper Epidermal Peels from Normal (W64A) and *gl15-m1* (W64A) Leaves.

(A) Leaf 4 from a W64A inbred plant representing the normal juvenile epidermal program. Juvenile epidermal cells are elongated, stain violet, and possess wavy cell walls. Veins appear as wide, blue-staining bands.

(B) Leaf 8 from a W64A inbred plant representing the normal adult epidermal program. The epidermis is highly differentiated into aqua-staining intercostal cells with invaginated cell walls, files of squarish purple bulliform cells (b) with an associated macrohair, and flanking prickle hairs (p). Also present are stomatal complexes and veins.

(C) and **(D)** Leaf 6 from a W64A inbred plant showing the normal juvenile-to-adult phase transition boundary. The arrows point to lineages of cells in the transition zone that appear to be affected by diffusible factors moving vertically **(C)** or horizontally **(D)** in the leaf. Staining can be seen to be very cell specific, with no diffusion beyond an individual cell boundary. b, bulliform cell.

(E) and **(F)** Leaf 4 from a *gl15-m1* (W64A) plant having a large revertant juvenile sector to the left in each photograph. **(E)** shows the leaf before peeling, and **(F)** shows the stained epidermal peel from the same leaf. An arrow in **(E)** points to the macrohair that appears in **(F)**. The epicuticular wax boundary in **(E)** appears to be coincident with the cell-specific color change in the stained cells in **(F)**. b, bulliform cell.

Bar in **(A)** = 100 μm for all panels. The orientation is with the tip of the leaf at top.

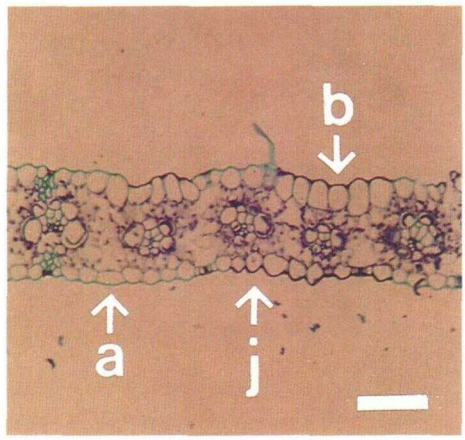

Figure 3. Transverse Cross-Section of a Sector Boundary from Leaf 4 of a *gl15-m1* Plant Stained with Toluidine Blue O.

Juvenile epidermal cells (j) are large, rounded, and possess dark purple cell walls. Bulliform cell (b) walls also stain purple but are taller and more rectangular than other cells. Adult cells (a) are small and rectangular with light blue cell walls. Bar = 100 μm.

phase transition traits listed in Table 1. Data in Table 2 show that *gl15-m1* plants, when compared to their normal siblings, had no significant differences in tiller formation, prop root production, the relative positions of the top ear, or the total number of vegetative nodes. In addition, the internal anatomy of *gl15-m1* leaves appeared to be uniform despite the presence of a sectored phenotype in the epidermal cell layers (Figure 3).

Interactions of *gl15-m1* with Other Maize Phase-Transition Mutants

In contrast to *gl15-m1*, which accelerates the onset of the epidermal adult phase, the *Cg1*, *Tp1*, *Tp2*, and *Hsf1-O* mutations delay the juvenile-to-adult vegetative phase transition and affect all phase transition traits (Galinat, 1966a; Poethig, 1988a; Bertrand-Garcia and Freeling, 1991). To determine how *gl15-m1* might interact with *Cg1*, *Tp1*, *Tp2*, and *Hsf1-O*, families segregating for double mutants were constructed and the phenotypes of the different genotypic classes examined. To control for the significant effects of environment and background genotype on the expression of all these genes, we took advantage of the variegated phenotype conditioned by the *gl15-m1* allele in the presence of an autonomous *Spm/En* element. Thus, in adjacent tissues of a single leaf, we could compare sectors in which *Gl15* was either inactive (transposon-inserted) or active (transposon-excised). The segregating families produced double mutants as well as single mutant and normal siblings, which were examined as controls. Plants homozygous for *gl15-m1* also segregated for *Spm/En*, resulting in mutable (*Spm/En* present) and stable (*Spm/En* absent) *gl15* phenotypes. The stable *gl15* phenotypes served as additional controls for the effects of *gl15* on the phenotypes of double mutants.

Within each segregating family, normal plants, semidominant single mutants, and *gl15-m1* plants all expressed their characteristic phase transition and reproductive phenotypes. The data for epidermal phase transition traits are presented schematically in Figure 4, and the results for nonepidermal traits are summarized in Table 2. The effect of the *gl15-m1* allele was confined to nodes 3 to 7 in a W64A background. When

Table 2. Nonepidermal Phase Transition Traits in Various Maize Genotypes

Genotype	No. of Plants	Number of Tillers		Nodes with Prop Roots		Top Ear[a] Node		Total Vegetative Nodes	
		Mode[b]	Range[b]	Mode	Range	Mode	Range	Mode	Range
W64A inbred[c]	12	0	All 0	7	8 to 6	12	12 to 11	17	17 to 16
gl15-m1 W64A[c]	13	0	All 0	6	7 to 6	12	12 to 11	17	17 to 16
W23 inbred	10	0	All 0	6	6 to 5	14	15 to 14	19	All 19
gl15-m1 W23	19	0	All 0	6	6 to 4	14	15 to 13	18	19 to 17
Cg1/+	10	7	9 to 3	10	12 to 5	ND[d]	24 to 17	25	31 to 24
Cg1/+ *gl15-m1*	12	6	9 to 4	9	12 to 5	ND[d]	20 to 16	25	28 to 23
Tp1/+	11	2	4 to 1	7	9 to 6	13	15 to 12	19	21 to 18
Tp1/+ *gl15-m1*	12	2	3 to 1	6	9 to 6	12	14 to 12	19	24 to 17
Tp2/+	9	1	3 to 0	9	10 to 8	15	15 to 13	22	24 to 19
Tp2/+ *gl15-m1*	9	1	3 to 0	9	10 to 7	14	16 to 13	21	25 to 20
Hsf1-O/+	12	1	3 to 1	8	9 to 8	13	14 to 13	17	18 to 17
Hsf1-O/+ *gl15-m1*	10	1	2 to 1	8	9 to 8	13	15 to 13	17	19 to 17

[a] Measured from the base of the plant.

[b] Modal values and ranges rather than means and standard deviations are reported because node numbers are discrete rather than continuous variables, and the range more accurately reflects the extent of variation observed for each trait.

[c] Data for normal and *gl15-m1* siblings within families segregating for double mutants are similar to those collected in a W64A background.

[d] ND, not determined. Most *Cg1*/+ plants failed to produce any fertile ears; the range is given only for those plants that did produce fertile ears.

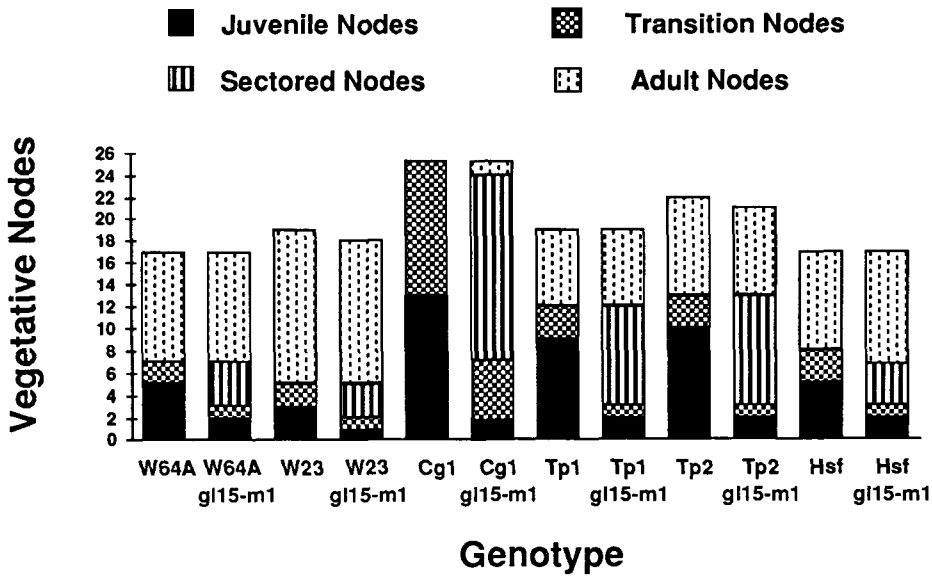

Figure 4. Schematic Representation of Whole-Plant and Epidermis-Specific Phenotypes Associated with the Maize Phase Transition Mutants.

Data represent the modal values from at least nine plants within each genotypic class. Black regions represent nodes producing juvenile epicuticular waxes and no epidermal macrohairs; white-stippled regions, nodes producing adult waxes and macrohairs; checkered regions, transition nodes expressing both juvenile and adult epidermal features; alternating black-and-white-striped regions, nodes showing defined somatic sectors. The height of each bar represents the total number of vegetative nodes.

examined in inbred line W23, however, where the juvenile-to-adult phase transition occurs in nodes 4 and 5, the *gl15-m1* phenotype was observed from the base of leaf 2 to node 5. Whereas normal plants within each double mutant family showed more variability than the W64A and W23 inbreds for each of the traits examined, the actual modal values for each normal sibling class fell within the ranges represented by the W64A and W23 inbreds (data not shown). Normal siblings therefore showed a transition from juvenile-to-adult growth in nodes 5 to 7 followed by 10 to 12 adult nodes. The *gl15-m1* plants in the double mutant families were phenotypically similar to those in a W64A background. Nodes 2 and 3 often showed a transition phenotype where juvenile as well as adult features were both present on the same leaf. From node 3 through node 7, *gl15-m1* plants showed a sectored phenotype followed by 10 to 12 adult nodes. Both normal and *gl15-m1* plants produced primary ear shoots at nodes 11 to 14.

The most severe extension of juvenile epidermal traits (18 nodes) was observed for *Cg1/+*, followed by *Tp2/+* (six nodes), *Tp1/+* (five nodes), and *Hsf1-O/+* (one node). Because *Cg1/+* plants had the most extreme juvenile phenotype, detailed results for this mutant are also presented in Figure 5. *Cg1/+* plants produced compressed nodes with narrow leaves and frequent tillers, showed prolonged expression of juvenile epidermal traits, such as waxes (Figure 5C), and either lacked or formed extremely vegetalized reproductive structures. Figure 5E shows that the upper epidermis from node 16 of a *Cg1/+* plant in an otherwise normal background had a mixture of adult and juvenile cell types, containing both violet-staining juvenile cells and adult-like bulliform cells. Normal plants, in contrast, exhibit a homogeneous adult phenotype at this node. Though not evident from the peel presented in Figure 5E, *Cg1/+* plants usually produced a few epidermal macrohairs after node 13 (Figure 4). Similar to *Cg1/+*, each of the other dominant mutants also extended the total number of nodes and nodes bearing tillers, prop roots, and the top ear (Table 2).

Figure 4 illustrates the effects of *gl15-m1* on epidermal traits in each of these dominant mutant backgrounds. Mutations at *Gl15* affected the same array of epidermal traits as they did in a normal background. Thus, beginning with node 3, adult epidermal traits appeared in each of the double mutants (e.g., the combination of *Cg1* with *gl15-m1* in Figure 5D). In *Cg1/+*, *Tp1/+*, and *Tp2/+* backgrounds, where juvenile traits are prolonged compared to normal plants, *gl15-m1* also displayed an extended mutable phenotype. Revertant juvenile sectors, normally confined to nodes 3 to 7, were observed beyond node 7 (e.g., Figure 5B) and were also present in leaves from lateral tillers. The position of these sectors coincided with the extension of the juvenile phase observed in plants carrying the *Cg1*, *Tp1*, and *Tp2* mutations alone. The size and frequency of such sectors also decreased with each successive node. *Hsf1-O/+ gl15-m1* double mutants showed no such extension of these sectors, suggesting at most an indirect interaction between *Hsf1-O* and *gl15-m1* in the genetic backgrounds examined. One peculiarity of the interaction of *gl15-m1* with *Cg1* was an intermediate epicuticular wax phenotype in nodes 3 to 7. Clonal

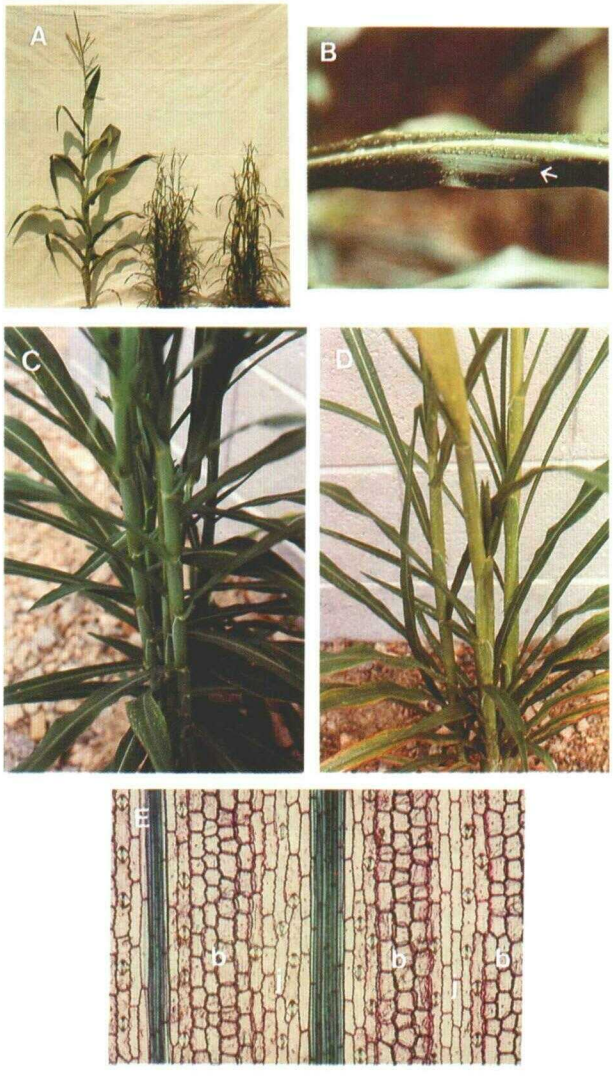

Figure 5. The *Cg1/+* and *Cg1/+ gl15-m1* Double Mutant Phenotypes.

(A) *gl15-m1* (left), *Cg1/+* (center), and *Cg1/+ gl15-m1* (right) plants at reproductive maturity.

(B) Leaf blade from node 17 of a *Cg1/+ gl15-m1* plant misted with water. The arrow indicates a somatic juvenile sector.

(C) Close-up of the vegetative morphology and constitutive juvenile epicuticular waxes associated with the *Cg1/+* genotype.

(D) Same view as shown in **(C)**, but of a *Cg1/+ gl15-m1* double mutant plant. Note the absence of juvenile epicuticular waxes but similarities in vegetative morphology to the *Cg1/+* sibling in **(C)**.

(E) Epidermal peel of the upper epidermis from node 16 of a *Cg1/+* leaf blade stained with toluidine blue O. Elongated juvenile epidermal cells (j) and squarish adult bulliform cells (b) are both present and are stained violet.

sectors were present but partially obscured by a diffuse pattern of juvenile waxes similar to those present at the juvenile-to-adult phase transition boundary (Figure 1F). Epidermal cells from nodes 3 to 7 of *Cg1/+ gl15-m1* plants also continued to express juvenile toluidine blue O staining characteristics and appeared similar to the epidermal peels from the transition region shown in Figure 2D. Beginning with nodes 8 and 9, *Cg1/+ gl15-m1* plants displayed the clearly defined wax sectors usually associated with *gl15-m1* (Figure 5B). Juvenile revertant sectors were observed even in the uppermost nodes of *Cg1/+ gl15-m1* double mutants. Stained epidermal peels from these sectored nodes appeared similar to the sector shown in Figure 2E. Somatic mutability for these traits has been previously reported for the *Cg1* mutation alone (Galinat, 1966b); however, we did not observe such phenotypes among the *Cg1/+* siblings examined. The unique effects of *Cg1* on *gl15-m1* suggested that *gl15-m1* interacts differently with *Cg1* compared to *Tp1*, *Tp2*, and *Hsf1-O*.

The *gl15-m1* mutation had no effect on the nonepidermal traits associated with the *Cg1*, *Tp1*, *Tp2*, and *Hsf1-O* mutations (Table 2). For example, the narrow leaves and increased number of tillers associated with the *Cg1* mutation were present in both *Cg1/+* and *Cg1/+ gl15-m1* backgrounds (Figure 5A). Maize leaves are known to exhibit differences in shape during development (Greyson et al., 1982). These changes in leaf shape are genotype dependent and have been difficult to correlate with any particular phase of vegetative growth, because adult leaves above the primary ear often have shapes similar to juvenile leaves. Figure 6 shows the variations in leaf shape, as measured by length/width ratio, for each genotype in a family segregating for *Cg1* and *gl15-m1*. Until the fourth node, leaves from each genotype became narrower. For both normal plants and *gl15-m1* siblings, leaf shape remained relatively constant from nodes 6 to 8 and then became progressively broader until the initiation of the tassel. In contrast, leaves from *Cg1/+* plants continued to show narrowing until node 16, after which comparisons could not be made to the other genotypes. The continued leaf narrowing associated with the *Cg1* mutation was

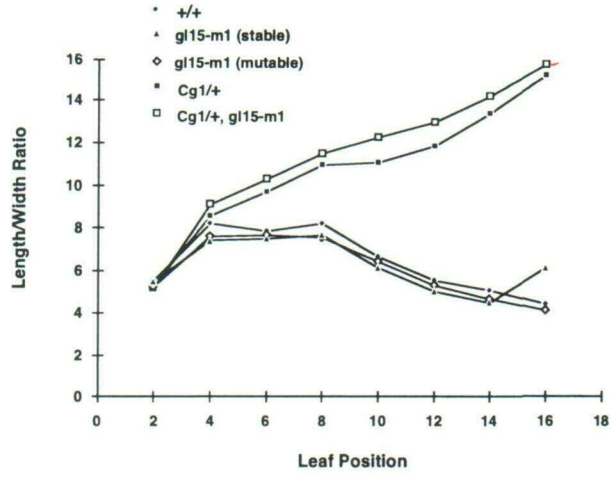

Figure 6. Morphology of Leaves from Normal, *Cg1/+*, *gl15-m1*, and *Cg1/+ gl15-m1* Siblings.

Leaf shapes are represented by leaf length/width ratios, and leaves are numbered from the base to the apex of the shoot.

not affected by *gl15-m1*. Because in normal plants juvenile nodes (2 to 4) show leaf narrowing whereas adult nodes exhibit leaf widening, we propose that leaf narrowing represents a juvenile trait. This hypothesis is supported by the fact that transition nodes in normal plants showed little change and that *Cg1*/+ plants, which express a constitutive juvenile phenotype, continue to show leaf narrowing. The *gl15-m1* mutation did not affect leaf shape in either normal or the *Cg1*/+ genetic backgrounds.

In summary, the phenotypes of double mutants demonstrated that *Gl15* activity is both sufficient and required for the ectopic expression of juvenile epidermal traits promoted by the action of the *Cg1*, *Tp1*, and *Tp2* mutant alleles. The *gl15-m1* mutation only affected epidermal traits and showed a spatial regulation of juvenile epidermal sectors in the presence of the dominant phase-transition mutants. These results support the conclusion that *Gl15* acts downstream of *Cg1*, *Tp1*, and *Tp2*.

DISCUSSION

Gl15 and the Maize Juvenile-to-Adult Phase Transition

The finding that *gl15-m1* alters the juvenile-to-adult vegetative phase transition offered the opportunity to define more precisely the mechanisms by which phase change occurs in maize. The loss-of-function *gl15-m1* mutation causes the replacement of adult for juvenile epidermal phenotypes. This observation suggests that the juvenile epidermal program is an acquired cell fate. This conclusion is supported by the presence of dominant, gain-of-function phase transition mutants that prolong juvenility. Two classes of mutations that would contradict this hypothesis are gain-of-function mutants that abbreviate the adult phase or loss-of-function mutants that condition juvenile instead of adult development. Such mutant phenotypes have not been identified in maize.

The *gl15-m1* mutation did not affect nonepidermal phase transition characters, but juvenile epidermal phenotypes within revertant sectors were dependent on the expression of the complete juvenile vegetative program. These observations demonstrated that in addition to *Gl15*, other factors act throughout the shoot to coordinate juvenile vegetative development. The nature of these other factors regulating phase change is currently not known. In his studies of *Cg1*, Galinat (1966b) suggested that the gradual process of phase change may reflect the effects of a physiological gradient. The polarized, nonclonal distribution of epidermal cell phenotypes at the normal phase transition boundary supports this hypothesis. It is possible that in adult nodes, a non-cell-autonomous "adult" factor(s) inactivates the juvenile program and *Gl15* function in the epidermis. However, the nature of the mutations known to affect phase change suggests that diffusible juvenility signals produced from the base of the shoot promote juvenile development. The gain-of-function *Tp1* and *Tp2* mutations are non-cell autonomous and acropetally extend the expression of all juvenile traits (Poethig, 1988b; Dudley and Poethig, 1993). Therefore, their wild-type gene products have been postulated to regulate the production or distribution of a diffusible "juvenile hormone" (Dudley and Poethig, 1993). Whereas *Cg1*, *Tp1*, and *Tp2* all appear to affect the supracellular mechanism(s) regulating phase change, it is not known if the normal (wild-type) alleles of these genes have any function. We have shown here that in all genetic backgrounds examined, *Gl15* is required for the expression of juvenile epidermal phenotypes beyond node 2. We therefore conclude that the supracellular juvenile program must interact with the *Gl15* gene product to control juvenile epidermal differentiation.

A model for the interaction of a shoot juvenility program with the cell-autonomous *Gl15* gene product is summarized

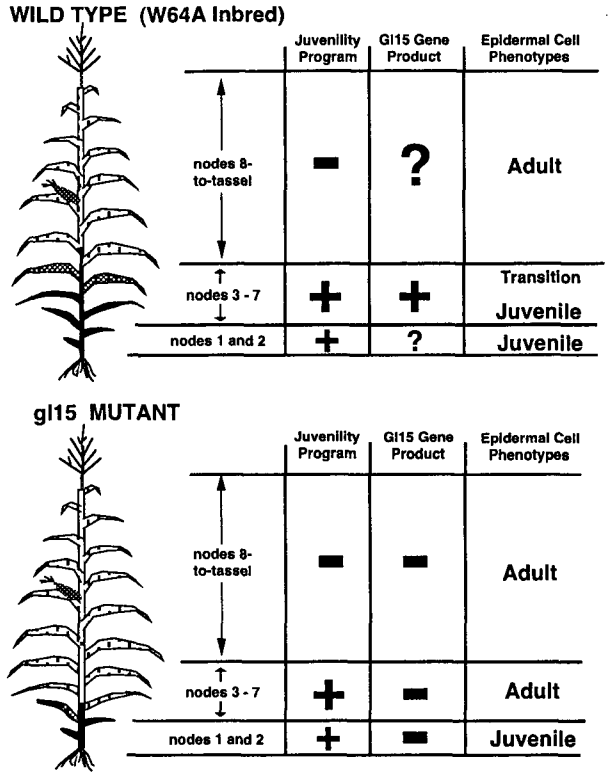

Figure 7. Model for the Role of *Gl15* in the Maize Epidermal Juvenile-to-Adult Phase Transition.

The proposed activities of the supracellular juvenility program and the cell-autonomous *Gl15* gene product with respect to positions within a normal plant (W64A inbred) and their observed epidermal phenotypes are shown at top. The observed effects of *gl15* mutations on epidermal cell phenotypes are summarized in a similar fashion at bottom. A (+) indicates the presence of a particular factor, whereas a (−) indicates its absence or significant reduction in that portion of the plant. Because *gl15* mutations have no phenotypic effects in nodes 1 and 2 or adult nodes, a (?) indicates that *Gl15* activity is not known for these nodes. Black-colored portions of the plants indicate the regions where homogeneous juvenile epidermal traits are found, shaded areas represent the transition regions, and white-stippled portions indicate the regions with homogeneous adult epidermal features.

in Figure 7. The first five nodes of normal plants (e.g., inbred W64A) express only juvenile traits, nodes 6 and 7 represent transition nodes, and nodes 8 to tassel exhibit only adult phenotypes. Somatic juvenile sectors resulting from the reactivation of *Gl15* activity also follow a similar pattern. These observations suggest that in normal plants the juvenile program operates in a positional gradient to promote the juvenile development of all cells within the first five nodes. The influence of the juvenile program gradually dissipates in an acropetal fashion beginning with node 6 until node 8, after which the shoot exhibits only adult characteristics. As discussed previously, the juvenile program may result from the presence of factors that condition juvenility or the absence of those that induce adult differentiation. Whatever the mechanism, it is not evident whether the *Gl15* gene product is present in the upper nodes of a normal maize plant, where its effects are not seen. The pattern of the transition from juvenile to adult epidermal characteristics in normal plants and the spatial regulation of *gl15-m1* revertant juvenile sectors are consistent with the action of a factor(s) operating upstream of *Gl15*. Currently, we do not know how this factor(s) might affect the expression of *Gl15* itself.

One prediction of the proposed model is that when either the juvenility program or the *Gl15* gene product is absent, adult epidermal traits are expressed. A clear exception to this proposed role for *Gl15* in phase transition is the juvenile character of maize leaves 1 and 2. In the genotypes we have examined, these nodes are not affected by *gl15* mutations. There are two likely explanations for this, both of which are also discussed in detail by Evans et al. (1994). One hypothesis is that all *gl15* mutant alleles represent hypomorphic rather than null mutations. The low level of gene activity associated with hypomorphic alleles could confer juvenile characteristics to leaves 1 and 2. An alternative possibility is that another gene product substitutes for *Gl15* in nodes 1 and 2. Evans et al. (1994) favor the second hypothesis because the two *gl15-Ref* alleles they examined showed similar phenotypes, and they asserted that the first two leaves are distinctly different from other juvenile leaves. Our data provide further support for the hypothesis that the juvenile phenotypes in leaves 1 and 2 are regulated independently from *Gl15*. The unique phenotype of nodes 3 to 7 in *Cg1/+ gl15-m1* double mutants suggests that if there is a second gene conferring juvenile traits to nodes 1 and 2, *Cg1* may extend its effects through node 7. Two predictions from this hypothesis are that *glossy* mutants could be identified in which the phenotype is confined to the first two leaves and that mutagenesis of *gl15* plants could produce plants that constitutively express adult epidermal characters.

Gl15 and Epidermal Cell Differentiation

Somatic transpositions from *gl15-m1* condition the presence of both *Gl15*-active and *Gl15*-inactive expression in adjacent tissues of a single leaf. This mosaic phenotype has provided insights into defining *Gl15* action during leaf development. The loss of *Gl15* activity coordinately accelerated the expression of adult-specific epidermal cell traits, apparently without affecting any other nonepidermal features of leaf development. The *gl15-m1* mutation therefore uncoupled epidermal differentiation from the overall vegetative juvenile-to-adult phase transition. Because we have not tested the effects of *gl15-m1* on phase-specific markers unique to internal cellular layers of the leaf (such as *Ragged leaves1*; Evans et al., 1994), we were not able to determine if *gl15-m1* alters phase transition in these tissues. The *gl15-m1* mutant phenotypes support the hypothesis that *Gl15* acts in an epidermal-specific manner. However, this is impossible to prove without molecular analysis of the distribution of the *Gl15* gene product. Even if *Gl15* is involved in the development of nonepidermal tissues, it is clear that this gene plays a major role in regulating leaf epidermal cell differentiation.

The epidermal tissue of transition nodes often simultaneously expresses both juvenile biochemical traits (e.g., waxes, violet toluidine blue O staining, and wavy cell walls) and an adult pattern of differentiation (e.g., bulliform cells and hairs). This observation indicates that these two epidermal phenotypes are not mutually exclusive cell fates and are regulated independently. In *gl15-m1* plants, the phenotypes of early versus late somatic transposition events provide further support for this hypothesis. Epidermal cells within small revertant sectors, which resulted from late transposition events, exhibited the biochemical juvenile epidermal traits (e.g., wax, wavy cell walls, and violet toluidine blue O staining) without disrupting bulliform cell or hair differentiation. In contrast, all adult cell types were absent in large sectors that arose from early transposition events, which included both epidermal cell layers. These observations suggest that the complete juvenile epidermal program involves the coordination of two independent events, the activation of juvenile biochemical phenotypes and the suppression of adult bulliform cell and hair formation. In nodes 3 to 7, *Gl15* activity is required for both of these functions during active epidermal cell divisions, because *gl15* mutations condition the coincident expression of adult epidermal phenotypes in both individual cells and the epidermal tissue as a whole.

Genetic mosaic analyses of the maize *liguleless1* (*lg1*) mutation have shown that the *Lg1* gene acts in internal tissues for the growth of auricles and in the epidermis for normal ligule development and differentiation of normal auricle epidermis (Becraft et al., 1990). Furthermore, wild-type *Lg1* function appears to transmit an inductive signal that is required for the proper organization of the epidermally derived ligular region (Becraft and Freeling, 1991). The phenotypic differences between small and large revertant sectors from *gl15-m1* suggest a similar mechanism involving *Gl15* may also serve to coordinate the two components of juvenile epidermal differentiation. Large sectors coincident in both epidermal layers probably include internal cells as well. Such sectors are completely juvenile. Smaller sectors appearing on only one side of the leaf exhibit juvenile cellular phenotypes without affecting bulliform cell or hair formation. These sectors may be specific to the epidermis. Thus, *Gl15* may act within internal cell layers of the leaf to suppress factors that induce adult epidermal differentiation (e.g., bulliform cells and hairs) as well as in the

epidermis to promote biochemical juvenile traits (e.g., waxes, wavy cell shape, and violet toluidine blue O staining). This hypothesis predicts that in transition nodes, which express both juvenile and adult traits, *Gl15* activity is confined to the epidermis. Genetic mosaic analyses capable of distinguishing epidermal versus internally derived *gl15* sectors combined with molecular studies of *Gl15* expression would directly address the role of intercellular communication in coordinating the epidermal phase transition.

Prospects

Analysis of the *gl15-m1* mutation has demonstrated that *Gl15* acts in a cell-autonomous fashion and is required for the expression of juvenile epidermal traits in nodes 3 through 7. Any proposed function for *Gl15* must take into account its apparent roles in both interacting with a supracellular juvenility program and coordinately directing the expression of epidermal genes that determine juvenile wax production, suppress bulliform cell and macrohair formation, and condition a juvenile cell wall composition and morphology. The *gl15* mutant phenotypes suggest that *Gl15* acts as a developmental regulator rather than as a direct participant in structural/synthetic functions. Adult phenotypes themselves do not appear altered by *gl15* mutations, only the relative time or position in which they are expressed. It is interesting to note that the *transparent testa glabrous* (*ttg*) mutation of Arabidopsis also affects the expression of two apparently unrelated epidermal traits, anthocyanins and trichomes (Lloyd et al., 1992). The existence of mutants such as *gl15* in maize, a monocot, and *ttg* in the dicot Arabidopsis suggests that the coordinate regulation of epidermal traits by single genes may also occur in other plant species.

The *dSpmI* element insertion in the *gl15-m1* allele should facilitate the isolation and molecular characterization of the *Gl15* gene product. Investigating the temporal and spatial expression patterns of *Gl15* in both normal and mutant plants will directly address our current hypotheses for its role in controlling the epidermal juvenile-to-adult phase transition in maize. Continued analyses of *Gl15* function should help elucidate the molecular mechanisms by which maize and other plants regulate changes in epicuticular wax production and cell wall characteristics as well as the transition from juvenile-to-adult vegetative development and its associated changes in cellular differentiation.

METHODS

Genetic Stocks and Crosses

The transposon mutagenesis stock from which the *gl15-m1*::*dSpm* allele was isolated was created by converting the *P-vv*::*Ac* and *r-sc:m3*::*Ds* alleles into a 4Co63 inbred background with C-sterile cytoplasm. The 4Co63 inbred stock used also contained active autonomous *En/Spm* elements (Peterson, 1990; Nelson, 1991; P.H. Sisco and S.P. Moose, unpublished observations). The mutagenesis stock was then open-pollinated as female by *r-sc:m3*::*Ds*–carrying males, and the subsequent F_1 ears were scored for single or multiple kernel sectors where *Ac* had transposed from *P-vv* yet was still present elsewhere in the genome (based on a red pericarp, purple-variegated aleurone phenotype). Single F_1 plants from 494 such sectors were selfed, and the F_2 progeny were screened for recessive mutations in the summer of 1990 in a Clayton, NC, nursery. The *gl15-m1* allele was recovered during this screen. All subsequent genetic crosses involving *gl15-m1* were conducted by hand pollination at one of three locations during 1990 to 1993: Clayton, NC, summer nursery; Homestead, FL, winter nursery; and Raleigh, NC, greenhouse.

We discovered that three reference alleles of *gl15* are maintained by the Maize Genetics Stock Center (Urbana, IL) based on searches through the pedigree records of the stock center (P. Stinard, personal communication). We have named these alleles *gl15-Sprague* (*gl15-S*), *gl15-Hayes* (*gl15-H*), and *gl15-Lambert* (*gl15-L*). The *gl15-S* allele was first reported in Sprague (1933) and the *gl15-H* allele in Hayes (1939); the *gl15-L* allele was discovered in the nursery of R.J. Lambert, University of Illinois, Urbana, and was subsequently identified as a *gl15* allele in Evans and Poethig (1991). The names of *gl15* itself and some of its alleles have varied through the years. When first reported by G.F. Sprague and H.K. Hayes, *gl15* was called "*gl4*." The numbering of the *glossy* genes was changed later (Anderson, 1955). Evans et al. (1994) called the *gl15-H* allele "*gl15-1*" and the *gl15-L* allele "*gl15-2*." They did not refer to the very first allele reported in 1933; this is the one we have called "*gl15-S*." An additional allele of *gl15* discovered in a *Mutator* transposon mutagenesis experiment has been designated *gl15-1106* in Evans et al. (1994). One verified stable derivative allele from *gl15-m1* has also been isolated in our laboratory. This allele was given a number according to the nursery row from which it was identified (*gl15-911625R*).

The *gl15-m1* allele was converted to a number of inbred lines by repeated backcrossing of variegated *gl15-m1* plants into the inbred background followed by selfing. For the phenotypic analyses presented in this article, we used progeny segregating for normal, *gl15*-variegated, and *gl15*-stable phenotypes that had been backcrossed three times to either the W64A or W23 inbred lines.

A TB-9Lc stock obtained from the Maize Genetics Stock Center was crossed as male onto *gl15-m1* plants. The TB-9Lc stock was heterozygous for a normal chromosome 9 carrying a wild-type *Gl15* allele and a B-A translocation with a break point 5 centimorgans proximal to *gl15* on chromosome 9 (Beckett, 1978). During the second microspore division, the B-9Lc chromosome frequently undergoes nondisjunction to produce gametes that are duplicated (*Gl15/Gl15*) or deficient (*0*) for most of the long arm of chromosome 9 (Beckett, 1978; Birchler and Alfenito, 1993). The resulting F_1 progeny segregated for normal (*Gl15/Gl15/gl15-m1* or *Gl15/gl15-m1*) and *gl15-m1/0* hypoploid genotypes. The hemizygous nature of the *gl15-m1/0* plants was confirmed by observations that they had thinner leaves characteristic of TB-9Lc hypoploids and ~50% pollen sterility.

Stocks heterozygous for *Cg1*, *Tp1*, *Tp2*, and *Hsf1-O* in a heterogeneous but mostly B73 inbred background were obtained from R. Bertrand (Colorado College, Colorado Springs). *Cg1/+*, *Hsf1-O/+*, and *Tp1/+* plants were selected on the basis of their mutant phenotypes and were backcrossed twice as males to the same *gl15-m1* stock in a W64A inbred background. Because *Tp2/+* plants rarely shed much pollen, selected *Tp2/+* plants were backcrossed twice as females with the same *gl15-m1* stock used above. As controls, normal F_1 plants were also selected and backcrossed by the *gl15-m1* parent. The resulting second backcross progeny segregated for the following genotypes:

M1+, *Gl15/gl15-m1* (*M* = semidominant mutant allele); *M1+*, *gl15-m1/gl15-m1*; *+/+*, *Gl15/gl15-m1*; and *+/+*, *gl15-m1/gl15-m1*. Genotypes were classified on the basis of phenotype and were verified by testcrossing representative plants to either the *gl15-H* stock or the W64A inbred. Three different segregating families were generated for each double mutant combination. Double mutant stocks that segregated for the above-mentioned four genotypic classes and for variegated and stable phenotypes among *gl15-m1* homozygotes were used in subsequent phenotypic analyses.

Phenotypic Analyses

Plants grown in either the summer nursery or greenhouse were observed and photographed throughout their development. For phenotypic analysis of families segregating for double mutants, 50 to 100 kernels from selected families were planted in adjacent rows at the Clayton, NC, nursery during the summer of 1993. Phenotypic data were recorded from at least nine plants within each genotypic class. Data were collected for all traits associated with juvenile versus adult phase change and are as follows: first leaf with epidermal hairs; last node with juvenile waxes; leaf shape (measured according to Poethig, 1988a); number and position of nodes (relative to the base of the shoot) bearing lateral tillers, adventitious roots, or the primary ear; and the total number of vegetative nodes. If any juvenile waxes or epidermal hairs were observed on either a leaf or sheath, that node was considered to express the trait. Hairs at leaf margins were not considered macrohairs because they were present at each leaf in most genotypes. For plants showing a variegated *gl15-m1* phenotype, the position of revertant somatic sectors relative to the base of the shoot was recorded. If even a tiny sector of juvenile epicuticular waxes was observed, that node was considered to express juvenile waxes.

Toluidine Blue O Staining of Maize Epidermal Peels

Epidermal peels were prepared from leaves of W64A, *gl15-m1*, *gl15-H*, *gl15-L*, and *gl15-S* plants. One-centimeter leaf sections were treated with a 1% cellulase (Onozuka R-10; Yakult Honsha, Tokyo) solution and stained with 0.05% toluidine blue O/acetic acid, pH 4.5, as described in Dudley and Poethig (1993). The stained peels were examined and photographed using a light microscope (Leitz Laborlux S, Wetzlar, Germany).

Light Microscopy

Leaf tissues from a fully expanded leaf 4 of *gl15-m1* plants that exhibited visible sectors of juvenile and adult epicuticular waxes were selected for histological analysis. Leaf sections were briefly vacuum infiltrated and fixed overnight at 4°C in a solution of 50% ethanol, 5% glacial acetic acid, and 10% formaldehyde in water. Samples were then dehydrated through an ethanol series and embedded in Paraplast paraffin embedding medium. Transverse 5-mm sections were rehydrated through an ethanol series and stained with 0.05% toluidine blue O/acetic acid, pH 4.5. Sections were examined and photographed using either a dissecting scope (Wild M3C, Heerbrugg, Switzerland) or a light microscope (Leitz Laborlux S).

ACKNOWLEDGMENTS

We thank William G. Brown, Jr., and Scott M. Furbeck, who initially discovered the *gl15-m1* plants in our transposon-tagging nursery and Bill Brown, who also assisted with the genetic crosses and phenotypic observations presented in this article. Maize stocks were from the Maize Genetics Stock Center (Urbana, IL) and generously provided by Irwin Greenblatt, Stephen Dellaporta, George F. Sprague, Sr., Peter A. Peterson, Ralph L. Bertrand, and Edward H. Coe, Jr. Ed Coe also provided a restriction fragment length polymorphism map for the region of chromosome 9 near the *gl15* locus. We also thank Robert D. Thomas, who assisted with the toluidine blue staining protocols and Wilma W.-L. Hu, who did the leaf cross-section appearing in Figure 3. We also gratefully acknowledge Matt Evans, Hilli Passas, and especially Scott Poethig for helpful discussions and for providing their manuscript prior to publication. This research was supported by U.S. Department of Agriculture Agricultural Research Service Current Research Information System Grant No. 6645-21000-006-00D and U.S. Department of Agriculture Cooperative State Research Service Competitive Grant No. 93-37304-8916. S.P.M was supported by a Graduate Fellowship in Plant Molecular Biology from the North Carolina Biotechnology Center, Research Triangle Park.

Received July 11, 1994; accepted August 10, 1994.

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