The Maize *Unstable factor for orange1* Is a Dominant Epigenetic Modifier of a Tissue Specifically Silent Allele of *pericarp color1*

Surinder Chopra,*,† Suzy M. Cocciolone,*,[1] Shaun Bushman,‡,[2] Vineet Sangar,† Michael D. McMullen§ and Thomas Peterson*,[3]

*Department of Zoology and Genetics and Department of Agronomy, Iowa State University, Ames, Iowa 50011, †Department of Crop & Soil Sciences, Pennsylvania State University, University Park, Pennsylvania 16802, ‡Genetics Area Program, University of Missouri, Columbia, Missouri 65211 and §Plant Genetics Research Unit, Agricultural Research Services, U.S. Department of Agriculture and the Plant Sciences Unit, University of Missouri, Columbia, Missouri 65211

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ABSTRACT

We have characterized *Unstable factor for orange1* (*Ufo1*), a dominant, allele-specific modifier of expression of the maize *pericarp color1* (*p1*) gene. The *p1* gene encodes an *Myb*-homologous transcriptional activator of genes required for biosynthesis of red phlobaphene pigments. The *P1-wr* allele specifies colorless kernel pericarp and red cobs, whereas *Ufo1* modifies *P1-wr* expression to confer pigmentation in kernel pericarp, as well as vegetative tissues, which normally do not accumulate significant amounts of phlobaphene pigments. In the presence of *Ufo1*, *P1-wr* transcript levels and transcription rate are increased in kernel pericarp. The *P1-wr* allele contains approximately six *p1* gene copies present in a hypermethylated and multicopy tandem array. In *P1-wr Ufo1* plants, methylation of *P1-wr* DNA sequences is reduced, whereas the methylation state of other repetitive genomic sequences was not detectably affected. The phenotypes produced by the interaction of *P1-wr* and *Ufo1* are unstable, exhibiting somatic mosaicism and variable penetrance. Moreover, the changes in *P1-wr* expression and methylation are not heritable: meiotic segregants that lack *Ufo1* revert to the normal *P1-wr* expression and methylation patterns. These results demonstrate the existence of a class of modifiers of gene expression whose effects are associated with transient changes in DNA methylation of specific loci.

PLANT genes involved in pigment biosynthetic pathways have been highly suitable for genetic studies because of the readily visible phenotypes (reviewed in Winkel-Shirley 2001). In maize, the synthesis of two broad categories of flavonoid pigments—anthocyanins and phlobaphenes—is controlled by a well-characterized set of regulatory and structural genes (Styles and Ceska 1977, 1989; Ludwig *et al.* 1990; Grotewold *et al.* 1991). The *pericarp color1* (*p1*) gene controls the synthesis of flavan-4-ol and phlobaphenes. The *p1* gene encodes a R2R3 MYB domain protein and directly regulates the transcription of structural genes required for phlobaphene biosynthesis (Grotewold *et al.* 1991, 1994). The pigmentation phenotypes specified by *p1* are most obvious in the kernel pericarp (seed coat) and cob glumes of mature maize ears. Many allelic forms of *p1* have been recognized and classified on the basis of their tissue-specific pigmentation (Brink and Styles 1966). Two well-characterized alleles that differ strikingly in their pericarp phenotype are *P1-wr* (*w*hite pericarp and *r*ed cob) and *P1-rr* (*r*ed pericarp and *r*ed cob). The phenotypic differences between *P1-wr* and *P1-rr* have been attributed to their differential transcriptional regulation (Chopra *et al.* 1996), which in turn may be a function of their unique gene structures. The *P1-rr* allele carries a single coding sequence (Grotewold *et al.* 1991) flanked by 5.2-kb direct repeat sequences, which contain regulatory elements (Sidorenko *et al.* 2000; Cocciolone *et al.* 2001). In contrast, *P1-wr* contains six copies of a 12.6-kb sequence containing the coding and presumptive *P1-wr* regulatory regions. The six *P1-wr* copies are arranged in direct orientation as a multicopy tandem repeat complex. Additionally, the *P1-wr* gene copies are hypermethylated in their coding and noncoding sequences regions relative to the *P1-rr* allele. On the basis of these observations, we previously proposed that the *P1-wr* multicopy structure may result in intraallelic interactions that give rise to silencing of *P1-wr* expression in kernel pericarp (Chopra *et al.* 1998).

Functional analysis of *P1-wr* promoter and coding sequences in transgenic maize plants, as well as studies of natural *p1* variants, have provided further support for the hypothesis that the organ-specific expression pattern of *P1-wr* is epigenetically regulated (Cocciolone *et al.* 2001). Other studies on regulation of maize flavo-

[1]*Present address:* Exseed Genetics, Ames, IA 50011.

[2]*Present address:* Department of Crop and Soil Science, Oregon State University, Corvallis, OR 97331.

[3]*Corresponding author:* Department of Zoology and Genetics, Department of Agronomy, Iowa State University, Ames, IA 50011-3260. E-mail: thomasp@iastate.edu

noid biosynthetic pathways have also led to the identification of epigenetic regulatory mechanisms (Das and Messing 1994; Dorweiler *et al.* 2000; Hoekenga *et al.* 2000; Hollick *et al.* 2000; Hollick and Chandler 2001). In plants, changes in epigenetic states are not associated with changes in DNA sequence, but are accompanied by alterations in DNA methylation (reviewed in Martienssen and Richards 1995; Kooter *et al.* 1999). Transcriptional gene silencing has been associated with increased DNA methylation and local chromatin compaction (Das and Messing 1994; Lund *et al.* 1995; Ye and Singer 1996). Additionally, homology-dependent gene silencing (HDGS) of plant transgenes has been associated with the presence of multiple transgene copies (reviewed in Flavell 1994; Matzke *et al.* 1996; Kooter *et al.* 1999). Phenomena similar to HDGS have been found in some cases of endogenous genes with multiple copies in Arabidopsis (Bender and Fink 1995), soybean (Todd and Vodkin 1996), and maize (Ronchi *et al.* 1995).

We report here the characterization of *Ufo1*, a factor that, in the presence of a *P1-wr* allele, induces striking kernel pericarp and plant pigmentation. The *Ufo1* factor by itself does not induce pigmentation, nor does it exhibit any detectable effects with alleles other than *P1-wr*. We show that *Ufo1* increases the levels of *P1-wr* transcripts and the rate of *P1-wr* transcription in pericarp nuclei. Moreover, *P1-wr* sequences exhibit reduced levels of DNA methylation in the presence of *Ufo1*. Interestingly, the activation of *P1-wr* by *Ufo1* is transient: the *P1-wr* expression and methylation patterns revert to their former state in progeny plants that lack *Ufo1*. We discuss these results in relation to current models of transcriptional gene silencing.

MATERIALS AND METHODS

Genetic stocks: A stock containing *P1-wr* and *Ufo1* was obtained from Derek Styles, University of Victoria (British Columbia, Canada). This stock was crossed with an inbred line 4Co63 of genotype *P1-ww c1 r-r*, the F_1 was self-pollinated, and F_2 progeny plants that were of *P1-ww* genotype were identified by their colorless tassel glume margins (Zhang *et al.* 2000). These *P1-ww* F_2 plants were outcrossed to a standard *P1-wr* inbred line, W23, and 20 plants were grown from each outcross. The resulting progeny plants that carried *Ufo1* were identified by orange pigmentation of leaf sheath, husk, and kernel pericarp. Subsequently, the *Ufo1* stock was maintained by repeated backcrossing to the W23 inbred line. The inbred line W23 (genotype *P1-wr c1 r-g*) and other inbred lines C123, B73, and W220 were obtained from the Maize Genetics Cooperation Stock Center (Urbana, IL). The *P1-ww* [4Co63] was obtained from the National Seed Storage Laboratory (Fort Collins, CO). The *P1-rr*4B2 and *P1-ww*1112 alleles used in this study have been previously described (Athma and Peterson 1991), as has allele *P1-rr*-CSF327 (Cocciolone *et al.* 2001).

DNA and RNA purification and Northern and Southern hybridization: Plant genomic DNA was prepared using a modified CTAB method (Sagai-Maroof *et al.* 1984). Restriction enzyme digestions were performed using enzymes, reagents, and incubation conditions from Promega (Madison, WI). Pericarp and cob glumes dissection, RNA extraction, poly(A)$^+$ RNA purification, and gel blotting was done as described previously (Chopra *et al.* 1996). Gel blots were stripped by washing for 15 min in boiling solution of 0.1% SDS before rehybridization. Plasmid DNA was prepared using the Maxi-prep DNA isolation kit (Promega). DNA fragments of *p1* used as probes have been described previously (Lechelt *et al.* 1989; Chopra *et al.* 1998; Cocciolone *et al.* 2001). The extent of genome-wide methylation was determined using repetitive DNA sequences as probes on blots carrying digests of genomic DNA from *P1-wr ufo1* and *P1-wr Ufo1* plants. These probes include p185 containing maize 185-bp knob repeat sequence (Ananiev *et al.* 1997), pMTY7SC1carrying maize telomeric sequence present near the maize *p1* gene on chromosome 1S (Gardiner *et al.* 1996), and pCT4.2 containing 5S ribosomal repeat sequences from Arabidopsis (Campell *et al.* 1992).

Nuclei isolation and run-on transcription assays: Nuclei were isolated from the kernel pericarps of ears sampled at 18 days after pollination (DAP). Pericarps were peeled from kernels and stored for up to 1 month at −20° in buffer containing 50% glycerol, 10 mm KCl, 10 mm $MgCl_2$, 0.1 mm dithiothreitol, and 20 mm 2-[*N*-morpholino]ethane sulfonic acid, pH 6.0. For isolation of nuclei, ~12–20 pericarps were removed from tissue storage buffer and gently blotted to remove excess buffer. Nuclei were prepared as previously described (Galbraith *et al.* 1983) by finely chopping the tissue in a plastic petri dish with a single-edged razor blade and filtering the cellular debris through 60- and 20-μm nylon filters. The nuclei were pelleted by centrifugation at 1000 rpm for 10 min at 4° (JS-4.3 swinging bucket rotor; Beckman Coulter, Fullerton, CA). After decanting the supernatant, the pellet was resuspended in nuclei storage buffer (50% glycerol, 5 mm $MgCl_2$, 50 mm Tris-HCl, pH 8.5). Alternatively, nuclei were isolated as described by Dorweiler *et al.* (2000), on the basis of the modified chromatin isolation protocol of Steinmuller and Apel (1986). All isolated nuclei were stored at −80°. Run-on transcription reactions were performed as described by Cone *et al.* (1993), using 5×10^6 to 8×10^6 nuclei per reaction. The reactions were treated with DNaseI (RNase-free, 27 μg per reaction) and proteinase K and extracted with phenol:chloroform:isoamyl alcohol (100:100:1). The labeled RNA was precipitated by adding 1/10 vol of 3 m sodium acetate, pH 5.0, and 2 vol of 100% ethyl alcohol. The supernatant was discarded and the pellet was briefly air dried and dissolved in 100 μl of H_2O. Samples were further purified by passage through a Micropure-EZ spin column (Millipore, Bedford, MA) and then through a Microcon 30 spin column (Millipore) according to the specifications of the manufacturers. The reaction products were hybridized to either DNA gel blots (Cone *et al.* 1986) or slot blots. For the DNA gel blots, 4 μg of plasmid DNA was digested to release an insert fragment. For slot blots, 100 ng of denatured purified gene fragments or the equivalent amount of linear plasmid vector DNA was added per slot and transferred to nitrocellulose membrane using a slot blotter. Plasmids used include: pWRP59, containing a 315-bp *P1-wr* cDNA (position +1080 to +1394) fragment inserted in pBluescript II SK(−) (Chopra *et al.* 1996); pC2, containing a maize *c2* gene cDNA (Wienand *et al.* 1986); pA1, containing a maize *a1* gene cDNA (Schwarz-Sommer *et al.* 1987); pChi, containing a maize *chi1* gene cDNA (Grotewold and Peterson 1994); and pZMU14, containing a genomic clone of the maize *ubiquitin* gene (Christensen *et al.* 1992). Results were quantified using phosphorimager and ImageQuant software (Molecular Dynamics, Sunnyvale, CA). Hybridization values for each gene were normalized by subtracting any background hybridization to plasmid DNA and dividing by the hybridization value of *ubiquitin*.

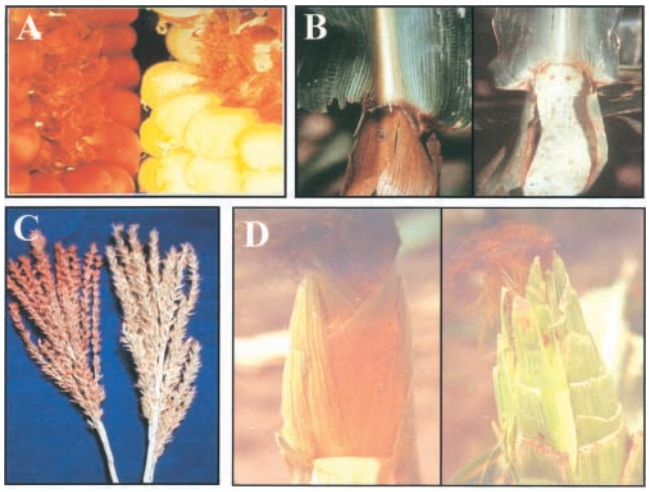

FIGURE 1.—Vegetative and floral organ pigmentation patterns of *P1-wr Ufo1* (left, A–D) and *P1-wr ufo1* (right, A–D). Mature ear (A), leaf blade and leaf sheath junction (B), tassel (C), silk and husk on an immature ear (D).

Mapping of *Ufo1*: Fifty-three backcross progeny from six families of (W23 *Ufo*1 × W23)BC_5 were grown in a greenhouse, and the *Ufo1* phenotype was scored visually on the basis of red pigmentation in the husk and kernel pericarp. DNA was extracted from lyophilized leaf tissue using the modified CTAB method. Methods and protocols of PCR amplification using simple sequence repeat (SSR) primers and DNA gel electrophoresis and blotting are described elsewhere (http://www.maizemap.org/resources.htm). The relative positions and linkage of polymorphic SSR markers were established using the three-point command of MAPMAKER/EXP, version 3.0b.

RESULTS

***Ufo1* is an allele-specific modifier of *p1* expression:** Charles Burnham isolated maize plants with variable orange plant color; these were subsequently shown to contain a modifier of *p1* expression that was named *Unstable factor for orange1* (*Ufo1*; STYLES 1982; STYLES *et al.* 1987). Genetic tests show that *Ufo1* is a dominant factor that segregates independently from *p1*. Plants that carry *P1-wr* and *Ufo1* have pigmented pericarp and more intense cob color compared with plants of genotype *P1-wr ufo1*, which have colorless pericarp and red cob glumes (Figure 1). In the pericarp, pigmentation is often strongest in and around the silk attachment point on the kernel. Additionally, plants of *P1-wr Ufo1* genotype commonly have intense pigmentation in other organs including dried silk, tassel glumes, husk, and leaf sheath (Figure 1). In addition to the gain of pigmentation, *P1-wr Ufo1* plants exhibit pleiotropic effects in which plants are variably stunted and weak. The enhanced pigmentation effect of *Ufo1* was observed in several inbred lines carrying *P1-wr*, including W22, W23, and B73. In contrast, *Ufo1* had no effect on pigmentation conferred by other *p1* alleles tested, including *P1-rr*, *P1-rw*, and *P1-ww*. A notable exception is *P1-rr*-CFS327, which shows variegated gain of pericarp pigmentation phenotype in the presence of *Ufo1* (see below). On the basis of these and previous genetic studies (STYLES *et al.* 1987), we hypothesized that *Ufo1* increases the levels of *P1-wr* expression and also induces ectopic expression of *P1-wr* in floral and vegetative tissues.

Map position of *Ufo1*: The *Ufo1* locus was genetically mapped in a population of 53 progeny derived from six families representing the fifth-generation backcross with inbred line W23. Progeny plants were scored for pigmentation phenotype and analyzed for genotype using SSR markers (MATERIALS AND METHODS). Twenty-three of the 53 backcross progeny expressed the *Ufo1* phenotype (Figure 2). Despite the possibility of variable expressivity and incomplete penetrance (see below), this ratio is not significantly different from the expected Mendelian segregation ratio of one locus determining *Ufo1* effects ($P = 0.55$). However, the difference between recombination ratios of the Ufo and the wild-type phenotypic classes for *umc1367* and *umc1179* markers provides evidence that some of the putative recombinants in the wild-type phenotypic class might be due to incomplete penetrance. Previously the maize genome was surveyed for linkage to *Ufo1*, and linked loci were found along the short arm of chromosome 10 (MATZ *et al.* 1991). Our data further narrow the location of the *Ufo1* locus to bin 3 on chromosome 10. Three-point analysis placed the *Ufo1* locus between *umc1576* and *umc1367* if all 53 individuals were included and coincident with *umc1367* if only the definitive Ufo expressers are considered.

***Ufo1* increases levels of *P1-wr* transcripts:** Previous studies have shown that *p1* directly regulates transcription of the maize *a1* gene that is required for phlobaphene pigment biosynthesis (GROTEWOLD *et al.* 1994). To test whether *Ufo1* acts by affecting *p1* transcript levels, we compared the levels of *p1* and *a1* transcripts in plants carrying *P1-wr Ufo1* with those with *P1-wr*, *P1-ww*, and *P1-rr* alleles in the absence of *Ufo1*. Steady-state transcript levels were determined by Northern analysis of poly(A)$^+$ RNA isolated from developing kernel pericarp. The blot was sequentially hybridized to radioactively labeled DNA fragments from the maize *p1*, *a1*, and *actin* genes (Figure 3A). Hybridization signals were quantified by densitometry and normalized to *actin* transcript levels. In agreement with our previous results (CHOPRA *et al.* 1996), *P1-wr* pericarp has reduced levels of *p1* and *a1* transcripts as compared with *P1-rr* pericarp. Pericarps of *P1-wr Ufo1* plants, however, have a threefold increase in *p1* transcripts and a twofold increase in *a1* transcripts relative to *P1-wr* pericarps (Figure 3B). Similar elevated transcript levels were also observed in other pigmented tissues of *P1-wr Ufo1* plants, including cob glumes, developing husk, and silks (data not shown). Thus, *Ufo1*-induced pigmentation of pericarp and other

A

Linkage analysis of *Ufo1* with (W23*Ufo1* x W23)BC_5 plants.

Marker	*umc1576*		*umc1367*		*umc1179*		*umc1336*	
Genotype	**A**	**H**	**A**	**H**	**A**	**H**	**A**	**H**
wildtype phenotype	9	21	23	8	20	9	17	13
Ufo phenotype	4	19	0	22	1	21	6	17

B

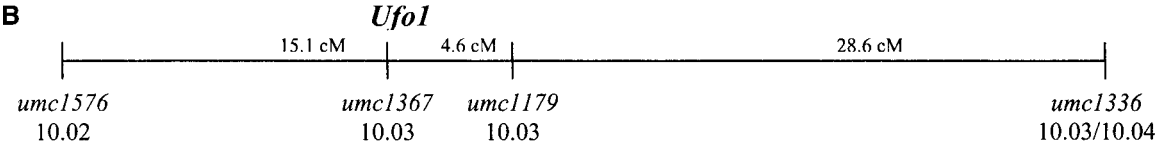

FIGURE 2.—Mapping of *Ufo1* on chromosome 10 of maize. (A) Number of individuals in each genotype/phenotype class for four markers in the (W23*Ufo1* × W23)BC_5 population. Under the genotype column A denotes plants homozygous for W23 alleles and H indicates that the plants were heterozygous for W23 and W23*Ufo1* alleles at that SSR locus. (B) Position of the *Ufo1* locus when only the 31 Ufo-expressing plants are considered. Markers are indicated in italics, with the chromosome and bin position underneath. For arrangement of SSR markers relative to maize chromosome 10 consensus restriction fragment length polymorphism markers, see the Missouri Maize Project web site (http://www.maizemap.org/maps.htm).

tissues is associated with increased *p1* and *a1* transcript levels.

To determine whether *Ufo1* affects *P1-wr* transcription rate or transcript stability, we performed nuclear run-on transcription assays using nuclei isolated from kernel pericarp of *P1-wr Ufo1*, *P1-wr*, *P1-ww*, and *P1-rr* genotypes. The labeled nascent transcripts were detected by hybridization to cDNA sequences immobilized on nylon membranes (MATERIALS AND METHODS). The genes tested included *c2*, *chi*, and *a1*, which encode enzymes for flavonoid pigment biosynthesis; a *P1-wr* cDNA fragment lacking the MYB domain to avoid cross-hybridization with other *Myb* genes; and a maize *actin* gene for normalization. The mean transcription rates determined in two experiments are shown in Figure 3C. The *P1-wr* and *P1-rr* alleles exhibit similar transcription rates, indicating that the reduced *P1-wr* steady-state transcript levels relative to *P1-rr* transcript levels (Figure 3A; CHOPRA *et al.* 1996) must be due to increased turnover of *P1-wr* transcripts relative to *P1-rr* transcripts; *i.e.*, *P1-wr* transcripts are less stable than *P1-rr* transcripts. In the case of *P1-wr Ufo1* pericarp, the transcription rate for *P1-wr* was on average threefold greater than that for *P1-wr* and *P1-rr* plants. Thus, presence of the *Ufo1* factor increases *P1-wr* transcription in pericarp. However, because *p1* steady-state transcript levels in *P1-wr Ufo1* pericarp are similar to those of *P1-rr* pericarp, *Ufo1* likely does not affect the rate of *P1-wr* message turnover. Thus, *Ufo1* increases *P1-wr* transcripts, but does not affect *P1-wr* transcript stability; the net effect is that levels of *P1-wr Ufo1* transcript levels and *P1-rr* transcript levels are approximately equal in kernel pericarp.

The increased level of *P1-wr* transcripts in *P1-wr Ufo1* compared with *P1-wr ufo1* is also associated with increased transcription rates of the *c2*, *chi*, and *a1* genes. This observation is consistent with the previously reported role of *p1* in activating transcription of these genes (GROTEWOLD *et al.* 1994). It is interesting that the rates of transcription of *c2*, *chi*, and *a1* are actually greater in *P1-wr Ufo1* than in *P1-rr* (Figure 3C), even though the steady-state levels of *P1-wr* transcripts in the presence of *Ufo1* are approximately equal to that of *P1-rr* (Figure 3B). It seems unlikely that *Ufo1* directly affects transcription of these genes independently of its action on *P1-wr*, because *Ufo1* does not enhance pigmentation in the presence of *P1-rr*. One possible explanation is that hypertranscription of *c2*, *chi*, and *a1* in the *P1-wr Ufo1* genotype reflects a higher activating potential of the P1-wr protein relative to the P1-rr protein; the proteins differ significantly at their C termini due to structural differences in the two genes, although there is no other evidence to suggest that they differ in their transcriptional activation potentials (CHOPRA *et al.* 1996). More surprising, despite a higher rate of *a1* transcription in the *P1-wr Ufo1* genotype relative to *P1-rr*, the steady-state levels of *a1* transcripts are similar in the *P1-wr Ufo1* and *P1-rr* genotypes. Thus, it seems that while *a1* transcription rate is increased in *P1-wr Ufo1*, the stability of *a1* transcripts is decreased. While there are a number of possible explanations for this observation, it would be interesting to determine whether increased flux through the flavonoid biosynthetic pathway, as in the case of *P1-wr Ufo1*, triggers a negative feedback mechanism that destabilizes the transcripts encoding enzymes of the pathway. Such a model could be tested by analysis of *P1-wr Ufo1* plants, which also carry a *c2* mutation that would block the first committed step of the flavonoid pathway.

***P1-wr* is demethylated in the presence of *Ufo1*:** In previous studies, we have shown that certain *P1-wr* coding sequences and flanking regions are resistant to cleavage by methylation-sensitive enzymes, while the corre-

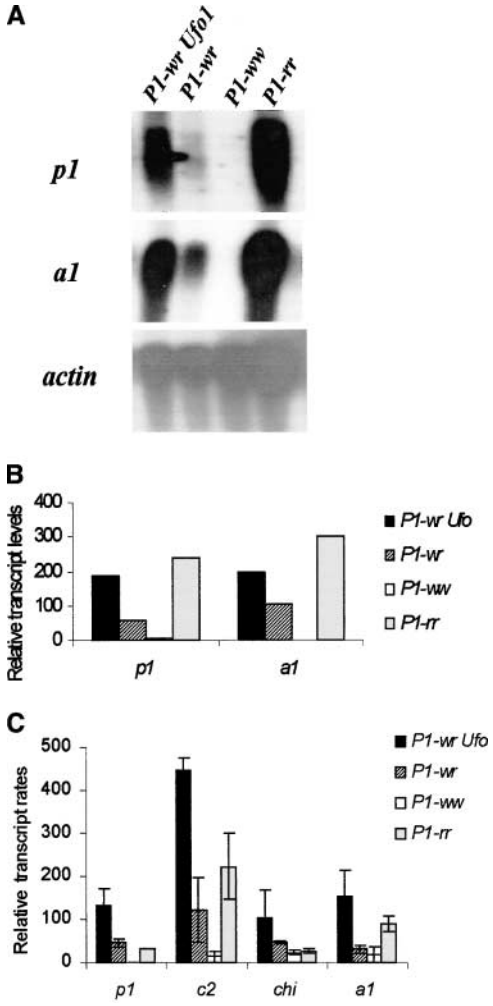

FIGURE 3.—Steady-state and nuclear run-on transcription analysis. (A) Northern analysis of *p1*, *a1*, and *actin* RNA levels from 18 DAP pericarp of *P1-wr Ufo1*, *P1-wr*, *P1-ww*, and *P1-rr* genotypes. Probes are indicated at the left. (B) Quantified RNA levels of *p1* and *a1* from hybridizations in A and normalized to *actin* to calculate relative transcript levels (*y*-axis). (C) Nuclear run-on assays. *In vitro* transcription data comparing the relative transcription rates of *p1*, *c2*, *chi*, and *a1* genes in 18 DAP pericarp from *P1-wr Ufo1*, *P1-wr*, *P1-ww*, *and P1-rr* genotypes. Results were quantified using a phosphorimager. Error bars represent the standard error values based on two independent experiments.

sponding sequences in *P1-rr* are readily cleaved (CHOPRA *et al.* 1998). These results indicate that many sequences in *P1-wr* are hypermethylated relative to *P1-rr*. Here, we tested whether the *Ufo1*-induced overexpression of *P1-wr* was associated with a change in *P1-wr* DNA methylation. Southern analysis of *P1-wr ufo1* and *P1-wr Ufo1* leaf genomic DNA digested with several restriction enzymes were hybridized to *p1* probe fragments (see Figure 4 for position of *p1* gene probe fragments). The hybridization patterns produced by *p1* probe fragment 15 are shown in Figure 4A. The absence of any restriction polymorphism between *P1-wr ufo1* and *P1-wr Ufo1* DNA digested with *Eco*RI, *Hin*dIII, *Kpn*I, and *Sac*I indicates that no changes in the gross *P1-wr* gene structure occurred in the presence of *Ufo1*. To test for alterations in DNA methylation, leaf DNA samples from *P1-wr ufo1* or *P1-wr Ufo1* plants were digested with the restriction enzyme isoschizomer pair *Msp*I and *Hpa*II, differing in their sensitivity to CG methylation. Southern hybridizations were performed using *p1* fragments that detect the entire 12.6 kb of each of the six *P1-wr* gene copies. The results of hybridization with three such fragments are shown in Figure 4B. The *p1* probe fragment 15 gives a similar hybridization pattern to *Msp*I digests of both *P1-wr Ufo1* and *P1-wr ufo1* DNA, with the exception of a 500-bp fragment, which is prominent in the *Ufo1* sample but absent from the *ufo1* DNA. *Hpa*II digestion, however, produces approximately seven fragments in *P1-wr Ufo1* DNA, which are absent in the *P1-wr ufo1* sample. The *p1* probe F-6 detects fragments of ~4.5 and 0.6 kb that are present in *Hpa*II digests of *P1-wr Ufo1*, but absent from *P1-wr ufo1*. Similarly, probe F-8B, which is part of the second intron of the *P1-wr* gene, detects fragments of 2.6 and 1.2 kb in *Hpa*II-digested *P1-wr Ufo1* DNA, whereas these fragments are absent or present at much-reduced levels in *P1-wr ufo1* DNA. Additionally, probe fragment F-13, which is derived from the third exon of *p1*, detects four fragments ranging in size from 1900 to 3600 bp in *Hpa*II-digested *P1-wr Ufo1* DNA, whereas probe F-13 detects a fragment of 8.1 kbp in *Hpa*II-digested *P1-wr ufo1* DNA (not shown). These results are summarized in Figure 5, which shows a comparison of the CpG methylation status of the *P1-wr Hpa*II sites in the presence or absence of *Ufo1*. These results indicate that most of the *Hpa*II sites in *P1-wr ufo1* are methylated and resistant to *Hpa*II digestion. In the presence of *Ufo1*, the *P1-wr* DNA is more sensitive to *Hpa*II digestion and therefore less methylated: some sites become fully demethylated, while other sites undergo partial demethylation. In contrast, we detected one site (Figure 5D, site 1) that shows partially increased methylation in the presence of *Ufo1*. Still other sites are unchanged in the presence or absence of *Ufo1*, remaining as methylated, demethylated, or partially methylated. The changes in methylation patterns occurred in both coding and noncoding regions of *P1-wr*. Due to the multicopy nature of the *P1-wr* allele, sites showing partial demethylation may reflect heterogeneity within the individual gene copies of the locus or among the different cells used to prepare DNA. Interestingly, sites that show complete sensitivity or resistance to *Hpa*II digestion must have the same methylation status among all of the six *P1-wr* copies; *i.e.*, they exhibit coordinate methylation throughout the *P1-wr* complex. In summary, the presence of *Ufo1* results in dramatic changes in the *P1-wr* methylation pattern, with most sites tested showing decreased methylation in the presence of *Ufo1*.

***Ufo1*-induced hypomethylation and overexpression of *P1-wr* are correlated:** Owing to the incomplete penetrance of the *Ufo1* factor, we tested whether *P1-wr* hypo-

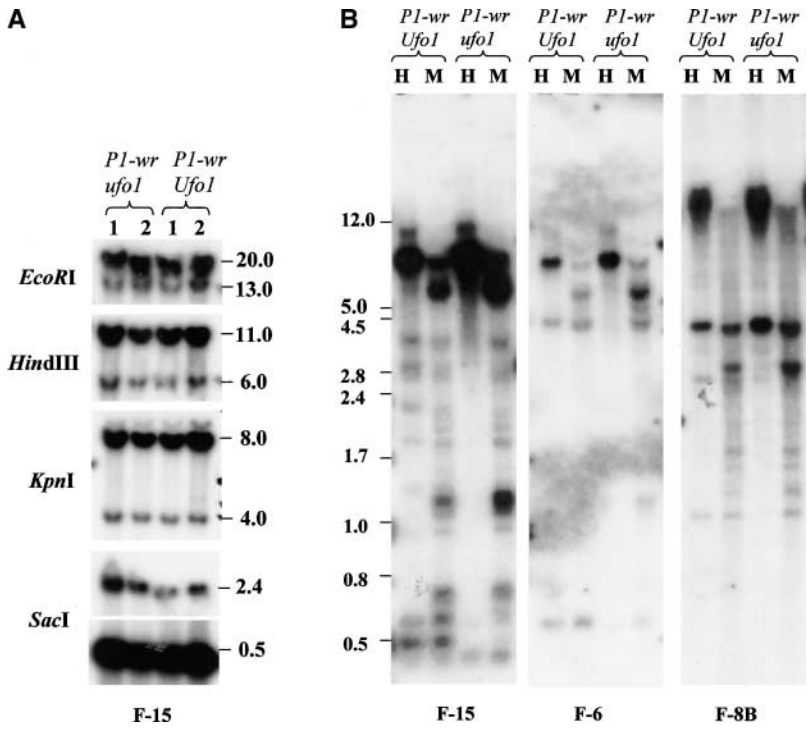

FIGURE 4.—Restriction mapping and DNA methylation analysis of *P1-wr* and *P1-wr Ufo1* plants. (A) Southern hybridization of genomic DNA isolated from two (1, 2) independent *P1-wr* and *P1-wr Ufo1* plants. The blot was hybridized to *p1* genomic DNA fragment 15 (LECHELT *et al.* 1989). Restriction enzymes are shown at left and hybridizing band sizes are indicated in kilobases at right. (B) Southern hybridization of genomic DNA digests of *P1-wr* and *P1-wr Ufo1* plants with *Hpa*II (H) and *Msp*I (M) restriction enzymes. Same blot was stripped and used to hybridize different DNA fragments of *p1* gene as probes. Results are shown for fragments 15, 6, and 8B. For position of each probe fragment see Figure 4. Molecular weight marker fragments (in kilobases) are shown at left.

methylation and enhanced pigmentation were completely correlated. Prior to this analysis, the original *Ufo1* stock was backcrossed four to five times with inbred lines W22 or W23 (both are *P1-wr*); this series of backcrosses was done to introgress *Ufo1* into a known genotype and thereby remove the possible influence of other genetic factors that may have contributed to the phenotypic variation in *Ufo1* expression. We then selected five parent plants with strong *Ufo1*-induced pigmentation in kernel pericarp and husk tissues; the ears of these plants were crossed with a *P1-wr* line, and their progeny were classified for Ufo expression on the basis of pigmentation of leaf sheath, husk, tassel glumes, pericarp, and cob glumes. Plants that showed pigmentation in tissues not normally pigmented in the recurrent inbred parent were classified as Ufo expressing, while plants that did

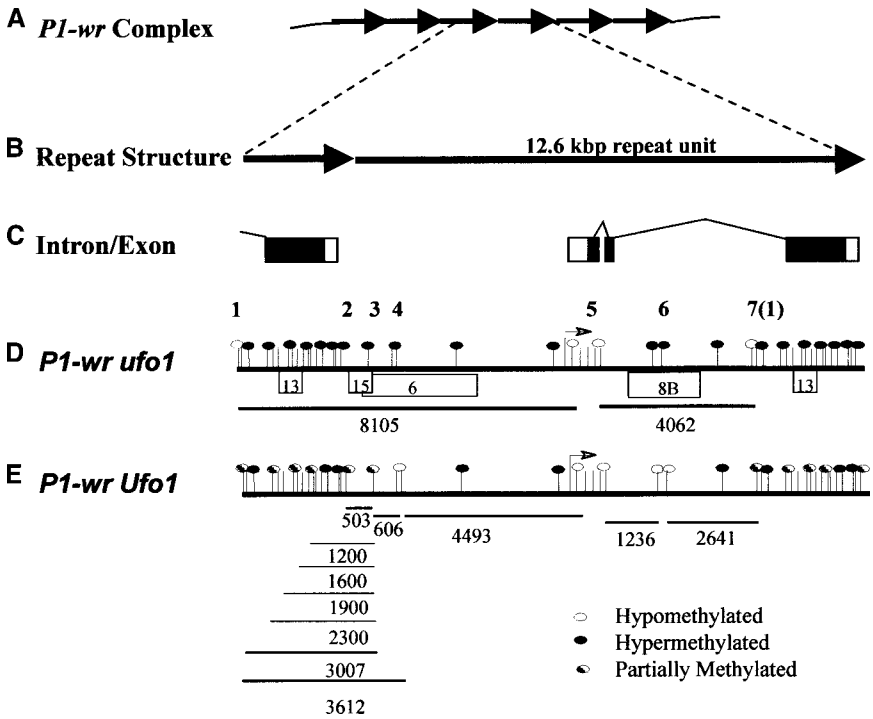

FIGURE 5.—DNA methylation of the *P1-wr* allele in *Ufo1* and *ufo1* plants. Cytosine methylation map deduced from Southern hybridization using *Msp*I and *Hpa*II digests of *P1-wr* and *P1-wr Ufo1* genomic DNA. (A) The structure of the 12.6-kb tandem repeats of *P1-wr.* (B) A full 12.6-kb repeat and a partial distal repeat are shown. (C) Intron/exon organization. Exons are depicted as solid boxes. Open boxes represent untranslated regions. A bent arrow in the methylation map diagram represents transcription start site. (D and E) Methylation maps of *P1-wr* and *P1-wr Ufo1* plant DNA. Numbers above the methylation map represent specific CpG sites discussed in the text. Restriction fragments generated by the *Hpa*II digest and probes (numbered boxes) used to map them are shown below the methylation map of *P1-wr.* Pattern of methylation states of *Hpa*II sites are shown as solid, open, and hatched circles.

not show any ectopic pigmentation were classified as nonexpressing (Table 1). In all progeny, the number of nonexpressing plants exceeded the number of expressing plants. Chi-square analysis showed that in two out of five backcross progeny, the ratio of Ufo-expressing *vs.* nonexpressing plants does not fit a 1:1 Mendelian ratio expected for a single dominant factor. In three out of the five families, the chi-square analysis does not cause rejection of a 1:1 ratio, but it is possible that testing of more individuals would likewise cause rejection of a 1:1 ratio. These results support the conclusion from the mapping data (see above) that *Ufo1* is incompletely penetrant.

A subset of plants from each backcross progeny was further tested for Ufo expression and *P1-wr* methylation status (Table 1). Genomic DNA was isolated from seedling leaves, digested with *Hpa*II, and used for DNA gel blots. The DNA gel blots were hybridized with *p1* probe F-15. The hybridization patterns were used to infer the cytosine methylation status of *P1-wr* in each plant. The seedling methylation status was then compared with the pigmentation state of each plant scored at maturity. The results show that the *P1-wr* methylation state and Ufo expression phenotype are completely correlated: all the expressing plants show hypomethylation, while the nonexpressing plants show hypermethylation of the *P1-wr* sequences. None of the plants belonged to the expressing and hypermethylated class or the nonexpressing and hypomethylated class. Hybridization results obtained from 17 progeny plants of backcross family 2 (Table 1) are presented in Figure 6. Lanes 1 and 2 contain DNA from *P1-ww Ufo1/-* and *P1-wr ufo1* plants, respectively. Eight plants with normal *P1-wr* pigmentation (R) had hybridization patterns similar to that of standard *P1-wr* (lane 2). In contrast, nine plants with enhanced pigmentation (U) showed enhanced sensitivity to digestions with *Hpa*II. The mature ears produced by the progeny plants showed considerable variation in pigmentation, ranging from darkly pigmented pericarp and cob (plant 12) to moderately variegated pericarp (plants 4 and 6) to a near-*P1-wr*-like pattern (plant 3). However, close examination shows that plants with a near-*P1-wr*-like pattern have small red sectors on kernels and husks (Figure 6, arrow lower left). Interestingly, the DNA gel blot hybridization patterns of such plants resemble that of the standard *P1-wr* allele except for the presence of a 503-bp fragment (Figure 6, lane 3). The 503-bp fragment is produced by digesting at hypomethylated *Hpa*II sites 2 and 3, located ~5 kbp 5′ of the *p1* transcription start site (Figure 5). Overall these results confirm that *Ufo1*-induced pigmentation is highly correlated with reduced methylation of *Hpa*II sites in the *P1-wr* gene complex.

Instability of *Ufo1* and its effects on *P1-wr*: To further investigate the inheritance and stability of the effects of *Ufo1* on *P1-wr* expression, we analyzed the selfed and outcross progeny of the four plants whose ears are pictured in Figure 6. Each of the four plants was self-pollinated and outcrossed to a standard inbred *P1-wr* line. Twenty seeds from each of the four self-pollinated ears and the corresponding outcrosses were grown to maturity. Genomic DNA was tested for methylation by *Hpa*II digestion, and plants were scored for ectopic pigmentation. Ear 3 (Figure 6) produced progeny plants, all of which were of standard *P1-wr* methylation and pigmentation pattern, indicating that the effect of *Ufo1* observed in the previous generations was now undetectable. Additionally, there were no Ufo-expressing plants in the progeny of the outcross to a standard *P1-wr* line [W23]. The *P1-wr* stock used for outcross with the *Ufo1* parent was naïve, *i.e.*, it had not previously been exposed to *Ufo1*, and hence it could not have become refractory to activation by *Ufo1*. We conclude that the incomplete penetrance of the *Ufo1*-induced activation of *P1-wr* results from loss of *Ufo1* function.

The self-pollinated ears 4, 6, and 12 produced *P1-wr Ufo1* and *P1-wr ufo1* plants in the ratio of 4:16, 6:14, and 6:14, respectively. These numbers do not fit the 3:1 ratio expected from segregation of a single dominant factor. The corresponding outcross progenies confirmed the presence of the *Ufo1* factor in these plants, although the numbers of *Ufo1* to *ufo1* plants again differed from a 1:1 ratio (data not shown). Many of the progeny ears produced by both self-pollination and outcross showed variegated or less extensive pigmentation compared with their parental ears. Similar to the results shown in Figure 6, the degree of hypomethylation observed by DNA gel blot analysis was strongly correlated with the intensity of pigmentation of mature plant tissues. Overall, these results indicate that *Ufo1* is highly unstable and spontaneously changes to a state that does not activate *P1-wr*. It is unknown whether *Ufo1* can become reactivated following its loss of function.

It is uncertain whether *Ufo1* can be maintained in a homozygous condition. Due to the high level of spontaneous inactivation, it has not been possible to demonstrate by progeny analysis that any individual plant is a *Ufo1* homozygote. However, we did not observe 25% kernel abortion or 25% seedling lethality in the progeny of self-pollinated *Ufo1* plants as would be expected if *Ufo1* were homozygous lethal. Some Ufo-expressing plants are severely stunted and died before maturity; whether these highly affected plants represent the *Ufo1* homozygous class could be determined by molecular analysis for inheritance of a linked marker, although this analysis has not yet been done.

***Ufo1* does not induce genome-wide demethylation:** To test whether *Ufo1* may affect methylation of other genomic sequences, DNA gel blots prepared from *Hpa*II- and *Msp*I-digested *P1-wr Ufo1* and *P1-wr ufo1* leaf DNA were hybridized to three probes that detect repetitive maize sequences: a 185-bp repeat sequence from a maize chromosomal knob; a 5S rDNA from Arabidopsis; and a fragment of maize subtelomeric sequence, which also

TABLE 1

***P1-wr Ufo1* × *P1-wr* backcross progeny analysis for Ufo expression and *P1-wr* DNA methylation**

Backcross	Inbred carrying *P1-wr* allele	Pericarp/cob pigmentation phenotype of ear parent of backcross progeny	Phenotypic classification for Ufo expressers *vs.* nonexpressers				Correlation of Ufo expression phenotype and *P1-wr* DNA methylation[a]				
			Total	Expressers[b]	Nonexpressers[c]	Chi-square probability[d]	Total	Expresser and hypermethylated	Expresser and hypomethylated	Nonexpresser and hypermethylated	Nonexpresser and hypomethylated
1. (*P1-wr Ufo1* × *P1-wr*)BC4	W22	Red/Red	205	71	134	$P < 0.001$	47	0	17	30	0
2. (*P1-wr Ufo1* × *P1-wr*)BC5	W23	Red/Red	49	18	31	$0.05 < P < 0.10$	25	0	10	15	0
3. (*P1-wr Ufo1* × *P1-wr*)BC4	W23	Red/Red	62	26	36	$0.20 < P < 0.30$	24	0	10	14	0
4. (*P1-wr Ufo1* × *P1-wr*)BC4	W23	Red/Red	58	15	43	$P < 0.001$	23	0	5	18	0
5. (*P1-wr Ufo1* × *P1-wr*)BC4	W23	Red/Red	70	30	40	$0.20 < P < 0.30$	30	0	12	18	0

[a] DNA methylation state of the *P1-wr* gene was determined for a subset of individual seedlings from each backcross progeny as described in text. Progeny were assigned to one of four classes on the basis of their Ufo expression phenotype and DNA methylation state.

[b] Expressers, no. of plants with increased pigmentation in kernel pericarp, cob, husk, and/or tassel glumes. This class includes plants that produced variegated husks and/or variegated pericarp.

[c] Nonexpressers, no. of plants with standard *P1-wr*-specified pigmentation pattern of colorless pericarp and red cob.

[d] Chi-square tests were performed on the phenotypic class data to test for 1:1 segregation ratios of Ufo expresser and nonexpresser plants.

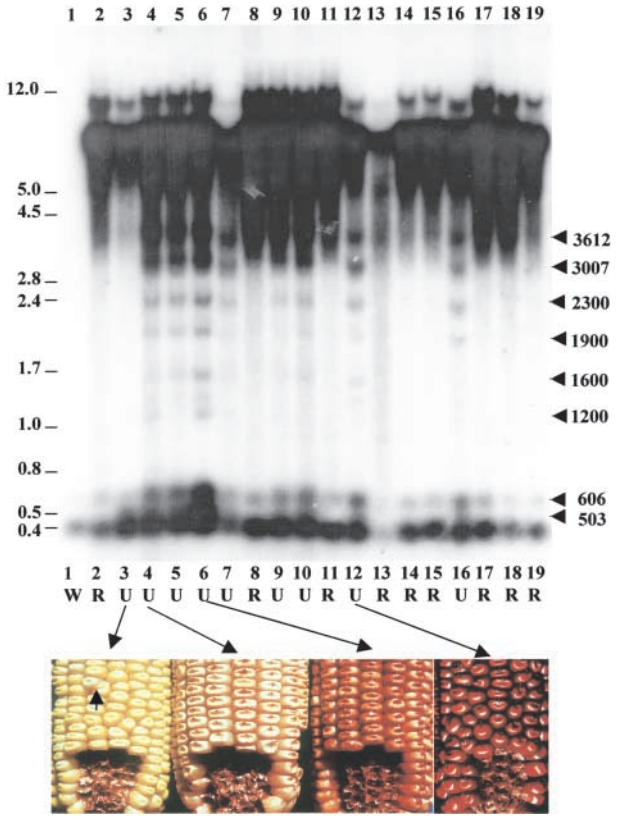

FIGURE 6.—Association of *P1-wr* methylation pattern and gain of pigmentation phenotype in the presence of *Ufo1*. Southern analysis of *Hpa*II-digested leaf genomic DNA of sibling progeny plants from a backcross (*P1-wr/P1-wr Ufo1/*− × *P1-wr/P-wr*) was hybridized to probe fragment 15. Standard DNA samples included are *P1-ww Ufo1* (W) and *P1-wr* (R). Ear phenotypes were scored as U and R for a Ufo expresser and for nonexpresser plants, respectively. Ear phenotypes of four sibling plants and their corresponding DNA lanes are indicated. Molecular weight marker fragments in kilobases are shown at left. Arrowheads with sizes in base pairs indicate major hypomethylated bands hybridizing in *P1-wr Ufo1* plant DNA.

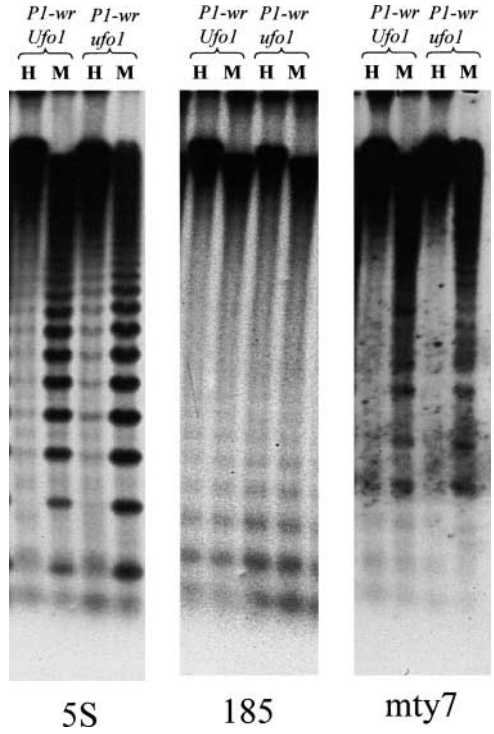

FIGURE 7.—*Ufo1* does not affect global DNA methylation. Gel blots prepared from *Hpa*II (H) and *Msp*I (M) digested *P1-wr Ufo1* and *P1-wr* leaf DNA were hybridized to repetitive probe DNA fragments: 5S rDNA from Arabidopsis (5S), maize 185-bp repeat sequence (185), and maize MTY7SC (mty7) repeat sequence.

cross-hybridizes with a repetitive sequence present near the maize *p1* gene on chromosome 1S. No detectable difference in methylation of these repeat sequences was observed between *P1-wr* and *P1-wr Ufo1* plants (Figure 7). We conclude that *Ufo1* does not induce genome-wide demethylation of repetitive sequences.

DISCUSSION

We describe here a dominant factor named *Ufo1* (STYLES *et al.* 1987), which modifies the organ-specific expression patterns of the *P1-wr* allele. This modifier was originally identified because of its ability to induce orange-red pigmentation in vegetative and floral tissues of maize plants (STYLES 1982). Our results confirm the finding by STYLES *et al.* (1987) that *Ufo1* alters pigmentation only in inbred lines that carry a specific allele of the *p1* gene, *P1-wr*. The *P1-wr* allele normally conditions a colorless kernel pericarp and red cob phenotype that is very common among United States Corn Belt Dent varieties (GOODMAN and BROWN 1988). A number of red-cobbed lines, including B37, B73, Oh43A, and W22, contain a multicopy *P1-wr* allele indistinguishable from the type found in W23 (S. CHOPRA, M. MCMULLEN and T. PETERSON, unpublished data). In addition to conferring cob pigmentation, *P1-wr* has been identified as a major quantitative trait locus (QTL) controlling levels of silk maysin, a C-glycosyl flavone compound with antibiosis activity against corn earworm larvae (BYRNE *et al.* 1996). Moreover, selection for grain yield in a population segregating multiple *p1* alleles resulted in significant increases in *P1-wr* allelic frequency (FRASCAROLI and LANDI 1998). Thus, the *P1-wr* allele is widespread, beneficial, and largely stable in its expression pattern. In contrast, the presence of *Ufo1* induces dramatic and variable alterations in *P1-wr* expression. For example, within a single *P1-wr Ufo/-* family, pericarp pigmentation can range from deep red through various degrees of variegated red to colorless. Husks are commonly variegated with prominent red and white sectors; variegation can also be observed on leaf sheath and tassel branches. Moreover, while the *P1-wr* expression pattern is stable in diverse genetic backgrounds, the *Ufo1*-induced pigmentation varies markedly in intensity and uniformity

in different genetic backgrounds. These observations suggest that additional genetic factors influence the interaction of *Ufo1* and *P1-wr*.

***Ufo1* induces *P1-wr* transcription:** The *P1-wr* locus contains six highly similar gene copies in a tandem direct array (Chopra *et al.* 1998), whereas the steady-state level of *P1-wr* transcripts in developing pericarp tissue is only ~30% of the level present in *P1-rr* pericarp (Figure 3; Chopra *et al.* 1996). If each of the six gene copies of *P1-wr* were equally expressed, then expression of each copy would be reduced to ~5% of the level of the single-copy *P1-rr* gene. However, run-on transcription analysis determined that the numbers of nascent *p1* transcripts are similar for the *P1-rr* and *P1-wr* alleles (Figure 3C). This suggests that the lower steady-state level of *P1-wr* transcripts is due to increased RNA turnover. In the presence of *Ufo1*, the transcription rate of *P1-wr* increases approximately threefold, and the steady-state levels of *P1-wr* transcripts also increase approximately threefold to a level approaching that of *P1-rr* pericarp. These results indicate that *Ufo1* increases the number of nascent transcripts of the *P1-wr* allele. Because *P1-wr* is multicopy, this increase could come about by increasing transcription among multiple copies or by activating a subset of silenced copies. In addition, these results demonstrate that upregulation of *P1-wr* RNA is sufficient to bring about a correlative gain of red pericarp pigmentation and enhanced cob color. This indicates that transcription of the structural genes required for phlobaphene pigmentation is limited by the level of *p1* expression. This conclusion is supported by previous observations that *P1-wr* homozygous plants have darker red cob color than plants in which *P1-wr* is heterozygous with a null *p1* allele (Brink 1958; Athma and Peterson 1991). Moreover, recent QTL studies have found that the *p1* locus has an additive effect on control of silk maysin levels (Byrne *et al.* 1996).

Variegated pigmentation and DNA demethylation in *P1-wr Ufo1* plants: Our genetic and molecular data show that the presence of *Ufo1*-induced pigmentation is strongly correlated with *P1-wr* overexpression and reduced methylation of *P1-wr* DNA. Each of the six *P1-wr* gene copies has the same pattern of methylation (Chopra *et al.* 1998). The *P1-rr* allele, which does not show any obvious interaction with *Ufo1*, is single copy and considerably less methylated than the *P1-wr* allele (Chopra *et al.* 1998). Further, we observed that the degree of enhanced pigmentation phenotype is strongly correlated with the degree of *P1-wr* demethylation. For example, a slight gain of *P1-wr* function indicated by a few red-striped kernels (Figure 6, ear 3) was associated with partial demethylation at two sites in *P1-wr* to release a 503-bp fragment upon digestion with *Hpa*II. Plants with strong pericarp pigmentation, however, exhibited a much greater extent of demethylation of *P1-wr* sequences (Figures 5 and 6). Previous studies have also identified a strong correlation of *p1* methylation and gene inactivation. In one study, an epiallele of *P1-rr* termed *P1-pr* exhibited suppressed pericarp pigmentation and reduced *p1* transcription. The *P1-pr* epiallele was associated with hypermethylation of sequences within a 1.2-kb enhancer fragment located 5 kb upstream of the transcription start site (Das and Messing 1994). A *DNA*seI-sensitivity assay showed that changes in chromatin conformation occurred within the 1.2-kb distal enhancer fragment of the suppressed (*P1-pr*) *vs.* active (*P1-rr*) allele of *p1* (Lund *et al.* 1995). In *P1-wr*, the sequences that would correspond to the 1.2-kb enhancer of *P1-rr* are truncated; however, two *Hpa*II sites border this truncated enhancer region in *P1-wr* (Figure 5, sites 2 and 3; Chopra *et al.* 1998). These two CpG sites are methylated in standard *P1-wr*, but they are demethylated in both the strong and the minimal Ufo1 individuals as evidenced by the appearance of the 503-bp *Hpa*II fragment (Figure 6, ear 3). Further investigation will be required to determine whether these sequences represent a critical *p1* regulatory region.

It is unclear whether the observed demethylation of *P1-wr* sequences is a direct effect of *Ufo1* or a secondary effect that results from activation of *P1-wr* transcription. A potential role of DNA methylation in gene silencing and HDGS has been demonstrated through the isolation and characterization of the *ddm1* and *hog1* mutations in Arabidopsis (Furner *et al.* 1998; Kakutani *et al.* 1999). The *DDM1* gene encodes a protein with homology to SWI2/SNF2-like proteins, and it has been suggested that it may affect DNA methylation by regulating chromatin structure (Jeddeloh *et al.* 1999). Unlike the *ddm1* mutation, our results indicate that *Ufo1* does not affect methylation of repetitive sequences other than *P1-wr* (Figure 7). Interestingly, recent analysis of the *mom1* mutation in Arabidopsis demonstrates the existence of an alternative type of epigenetic regulation that mediates transcriptional silencing independently of changes in DNA methylation (Amedeo *et al.* 2000).

Allele specificity of the *p1-Ufo1* interaction: A striking feature of *Ufo1* is its allele-specific effects on pigmentation. As noted above, *Ufo1*-induced pigmentation was observed only with *P1-wr* alleles, with the exception of a novel allele termed *P1-rrCFS327*. This latter allele normally specifies grainy red pericarp and red cob. However, *P1-rrCFS327* gives distinct pericarp, husk, and vegetative tissue pigmentation in the presence of *Ufo1*. Interestingly, *P1-rrCFS327* has a multicopy gene structure resembling that of standard *P1-wr*, but which is hypomethylated relative to standard *P1-wr*. In other words, *P1-rrCFS327* appears to be a semistable epiallele of *P1-wr* with reduced methylation and increased expression of *P1-wr* (Cocciolone *et al.* 2001). Thus, *Ufo1* may affect only *p1* alleles that have a multicopy structure. Alternatively, the allele specificity of *Ufo1* could reflect sequence differences in the *P1-rr* and *P1-wr* alleles located >5 kb upstream and 8 kb downstream of the transcription start site.

Allele-specific differences were also observed in a study of *p1* paramutation: *P1-rr* expression is suppressed by exposure to a transgene locus that carries a 1.2-kb enhancer fragment derived from the *P1-rr* allele. Moreover, the suppressed state is associated with *P1-rr* hypermethylation (Sidorenko and Peterson 2001). In contrast, *P1-wr* is not affected by exposure to the paramutagenic locus, nor does *P1-wr* transmit a paramutagenic signal (Sidorenko and Peterson 2001). Thus, the *P1-rr* and *P1-wr* alleles exhibit distinct and complementary susceptibilities to *Ufo1* activation and transgene-induced paramutation.

Pleiotropism and possible function of *Ufo1*: As mentioned above, *P1-wr Ufo1* plants exhibit variable defects in growth and vigor. The degree of stunted growth is proportional to the intensity of plant pigmentation, leading to an earlier suggestion that production of phlobaphene pigments in vegetative tissues where they do not normally accumulate may be deleterious to the plants (Styles *et al.* 1987). This idea is supported by the observation that transgenic maize plants that express *p1* transgenes in vegetative tissues show similar deleterious effects (Cocciolone *et al.* 2001; S. M. Cocciolone, unpublished data). In addition to its effects on *P1-wr*, *Ufo1* could conceivably affect other genes whose altered expression may lead to other pleiotropic effects. Pleiotropic developmental abnormalities have also been observed in maize plants carrying mutations in *mediator of paramutation1* (*mop1*), which is involved in establishment and maintenance of paramutation at several maize loci required for anthocyanin biosynthesis (Dorweiler *et al.* 2000). The reported pleiotropic effects of *mop1* include short stature, delayed flowering, and tassel feminization, whereas *Ufo1* seems to affect primarily plant stature. Like *Ufo1,* the *mop1* mutation does not elicit global genome demethylation (Dorweiler *et al.* 2000), but it does lead to demethylation of silenced *Mutator* transposon sequences (Lisch *et al.* 2002). It will be interesting to determine whether *Ufo1* may similarly affect *Mutator* transposon methylation.

We considered the possibility that *ufo1* encodes or controls a *trans*-acting factor whose normal function is to activate *p1* expression in floral organs. In this model, the *Ufo1* allele would represent a hypermorph that is overexpressed in floral organs and ectopically expressed in vegetative tissues. This model seems unlikely due to the allele specificity of *Ufo1* action. The *P1-rr* and *P1-wr* coding and regulatory sequences are highly similar: in the promoter region, *P1-rr* and *P1-wr* are 99% identical over a 5-kb region 5′ of the transcription start sites. Thus, it seems likely that *P1-rr* and *P1-wr* would be activated by the same regulatory factors. Yet, *Ufo1* has no detectable effect on expression of a standard *P1-rr* allele, even though enhancement of *P1-rr* expression could easily have been observed in husks and vegetative tissues of *P1-rr* plants.

The results of this and previous studies indicate that transcription of the *P1-wr* gene complex is suppressed in kernel pericarp and that this suppressed state is associated with *P1-wr* hypermethylation. We propose that *Ufo1* alleviates the transcriptional suppression of *P1-wr*; the associated demethylation of *P1-wr* sequences may be a secondary effect of transcriptional activation. *Ufo1* may encode or regulate a factor that modifies the chromatin structure of *P1-wr* and possibly other multicopy complex loci. Genes that putatively affect chromatin structure have been mapped recently in maize (http://www.chromdb.org). Two of these genes map in the vicinity of *Ufo1* in bin 10.03, chromosome 10: *sdg108b* is related to SET domain genes, some of which encode histone methyltransferases (Rea *et al.* 2000), and *chr109a* is related to chromatin-remodeling complex subunit R (SWI2/SNF2; http://www.chromdb.org). Due to the highly unstable nature of *Ufo1*, it would be impractical to use transposon tagging for its molecular isolation; hence it will be interesting to determine whether *Ufo1* represents a mutant allele of one of these candidate genes. Additionally, it will be important to test whether *Ufo1* can activate the expression of other maize genes, both natural and transgenic, that contain multicopy repeat sequences.

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LITERATURE CITED

Amedeo, P., Y. Habu, K. Afsar, O. Mittelsten Scheid and J. Paszkowski, 2000 Disruption of the plant gene MOM releases transcriptional silencing of methylated genes. Nature **405:** 203–206.

Ananiev, E. V., O. Riera-Lizarajzu, H. W. Rines and R. L. Phillips, 1997 Oat-maize chromosome addition lines: a new system for mapping the maize genome. Proc. Natl. Acad. Sci. USA **94:** 3524–3529.

Athma, P., and T. Peterson, 1991 Ac induces homologous recombination at the maize P locus. Genetics **128:** 163–173.

Bender, J., and G. R. Fink, 1995 Epigenetic control of an endogenous gene family is revealed by a novel blue fluorescent mutant of Arabidopsis. Cell **83:** 725–734.

Brink, R. A., 1958 A stable somatic mutation to colorless from variegated pericarp in maize. Genetics **37:** 519–544.

Brink, R. A., and E. D. Styles, 1966 A collection of pericarp factors. Maize Genet. Coop. News Lett. **40:** 149–160.

Byrne, P. F., L. L. Darrah, M. E. Snook, B. R. Wiseman, N. W. Widstrom *et al.*, 1996 Maize silk-browning, maysin content, and antibiosis to the corn earworm, *Helicoverpa zea* (Boddie). Maydica **41:** 13–18.

Campell, B. R., Y. Song, T. E. Posch, C. A. Cullis and C. D. Town, 1992 Sequence and organization of 5S ribosomal RNA-encoding genes of *Arabidopsis thaliana.* Gene **112:** 225–228.

Chopra, S., P. Athma and T. Peterson, 1996 Alleles of the maize *p* gene with distinct tissue specificities encode *myb*-homologous proteins with distinct tissue specificities. Plant Cell **8:** 1149–1158.

Chopra, S., P. Athma, X. G. Li and T. Peterson, 1998 A maize Myb homolog is encoded by a multicopy gene complex. Mol. Gen. Genet. **260:** 372–380.

Christensen, A. H., R. A. Sharrock and P. H. Quail, 1992 Maize polyubiquitin genes: structure, thermal perturbation of expression and transcript splicing, and promoter activity following transfer to protoplasts by electroporation. Plant Mol. Biol. **18:** 675–689.

Cocciolone, S. M., S. Chopra, S. A. Flint-Garcia, M. D. McMullen and T. Peterson, 2001 Tissue-specific patterns of a maize Myb transcription factor are epigenetically regulated. Plant J. **27:** 467–478.

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

Cone, K. C., S. M. Cocciolone, F. A. Burr and B. Burr, 1993 The maize anthocyanin regulatory gene pl is a duplicate of c1 that functions in the plant. Plant Cell **5:** 1795–1805.

Das, O. P., and J. Messing, 1994 Variegated phenotype and developmental methylation changes of a maize allele originating from epimutation. Genetics **136:** 1121–1141.

Dorweiler, J. E., C. C. Carey, K. M. Kubo, J. Hollick, J. L. Kermicle *et al.*, 2000 Mediator of paramutation 1 (mop1) is required for the establishment and maintenance of paramutation at multiple maize loci. Plant Cell **12:** 2101–2118.

Flavell, R. B., 1994 Inactivation of gene expression in plants as a consequence of specific sequence duplication. Proc. Natl. Acad. Sci. USA **91:** 3490–3496.

Frascaroli, E., and P. Landi, 1998 Allelic frequency change of P1 gene in a maize population after recurrent selection for grain yield. Crop Sci. **38:** 1391–1394.

Furner, I. J., M. A. Sheikh and C. E. Collette, 1998 Gene silencing and homology-dependent gene silencing in Arabidopsis: genetic modifiers and DNA methylation. Genetics **149:** 651–662.

Galbraith, D. W., K. R. Harkins, J. R. Maddox, N. M. Ayres, D. P. Sharma *et al.*, 1983 Rapid flow cytometric analysis of the cell cycle in intact plant tissues. Science **220:** 1049–1051.

Gardiner, J., E. Coe and S. Chao, 1996 Cloning maize telomeres by complementation in *Saccharomyces cerevisiae*. Genome **39:** 736–748.

Goodman, M. M., and W. L. Brown, 1988 Races of corn, pp. 33–79 in *Corn and Corn Improvement*, edited by G. F. Sprague and J. W. Dudley. American Society of Agronomy, Madison, WI.

Grotewold, E., and T. Peterson, 1994 Isolation and characterization of a maize gene encoding chalcone flavanone isomerase. Mol. Gen. Genet. **242:** 1–8.

Grotewold, E., P. Athma and T. Peterson, 1991 Alternatively spliced products of the maize P gene encode proteins with homology to the DNA-binding domain of myb-like transcription factors. Proc. Natl. Acad. Sci. USA **88:** 4587–4591.

Grotewold, E., B. J. Drummond, B. Bowen and T. Peterson, 1994 The myb-homologous P gene controls phlobaphene pigmentation in maize floral organs by directly activating a flavonoid biosynthetic gene subset. Cell **76:** 543–553.

Hoekenga, O. A., M. G. Muszynski and K. C. Cone, 2000 Developmental patterns of chromatin structure and DNA methylation responsible for epigenetic expression of a maize regulatory gene. Genetics **155:** 1889–1902.

Hollick, J. B., and V. L. Chandler, 2001 Genetic factors required to maintain repression of a paramutagenic maize *pl1* allele. Genetics **157:** 369–378.

Hollick, J. B., G. I. Patterson, I. M. Asmundsson and V. L. Chandler, 2000 Paramutation alters regulatory control of the maize pl locus. Genetics **154:** 1827–1838.

Jeddeloh, J. A., T. L. Stokes and E. J. Richards, 1999 Maintenance of genomic methylation requires a SWI2/SNF2-like protein. Nat. Genet. **22:** 94–97.

Kakutani, T., K. Munakata, E. J. Richards and H. Hirochika, 1999 Meiotically and mitotically stable inheritance of DNA hypomethylation induced by ddm1 mutation of *Arabidopsis thaliana*. Genetics **151:** 831–838.

Kooter, J. M., M. A. Matzke and P. Meyer, 1999 Listening to the silent genes: transgene silencing, gene regulation and pathogen control. Trends Genet. **4:** 340–347.

Lechelt, C., T. Peterson, A. Laird, J. Chen and S. Dellaporta, 1989 Isolation and molecular analysis of the maize *P* locus. Mol. Gen. Genet. **219:** 225–234.

Lisch, D., C. C. Carey, J. E. Dorweiler and V. L. Chandler, 2002 A mutation that prevents paramutation in maize also reverses *Mutator* transposon methylation and silencing. Proc. Natl. Acad. Sci. USA **99:** 6130–6135.

Ludwig, S. R., B. Bowen, L. Beach and S. R. Wessler, 1990 A regulatory gene as a novel visible marker for maize transformation. Science **247:** 449–450.

Lund, G., O. P. Das and J. Messing, 1995 Tissue-specific DNase I-sensitive sites of the maize P gene and their changes upon epimutation. Plant J. **7:** 797–807.

Martienssen, R. A., and E. J. Richards, 1995 DNA methylation in eukaryotes. Curr. Opin. Genet. Dev. **5:** 234–242.

Matz, E. C., F. Burr and B. Burr, 1991 Mapping new mutations using RFLPs. Maize Genet. Coop. News Lett. **65:** 164.

Matzke, M. A., A. J. M. Matzke and W. B. Eggleston, 1996 Paramutation and transgene silencing: A common response to invasive DNA? Trends Plant Sci. **1:** 382–388.

Rea, S., F. Eisenhaber, D. O'Carroll, B. D. Strahl, Z. W. Sun *et al.*, 2000 Regulation of chromatin structure by site-specific histone H3 methyltransferases. Nature **406:** 593–599.

Ronchi, A., K. Petroni and C. Tonelli, 1995 The reduced expression of endogenous duplications (REED) in the maize *R* gene family is mediated by DNA methylation. EMBO J. **14:** 5318–5328.

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

Schwarz-Sommer, Z., N. Shepherd, E. Tacke, A. Gierl, W. Rohde *et al.*, 1987 Influence of transposable elements on the structure and function of the A1 gene of Zea mays. EMBO J. **6:** 287–294.

Sidorenko, L., and T. Peterson, 2001 Transgene-induced silencing identifies sequences involved in the establishment of paramutation of the maize *p1* gene. Plant Cell **13:** 319–335.

Sidorenko, L. V., X. Li, S. M. Cocciolone, S. Chopra, L. Tagliani *et al.*, 2000 Complex structure of a maize Myb gene promoter: functional analysis in transgenic plants. Plant J. **22:** 471–482.

Steinmuller, K., and K. Apel, 1986 A simple and efficient procedure for isolating plant chromatin which is suitable for studies of DNase I–sensitive domains and hypersensitive sites. Plant Mol. Biol. **7:** 87–94.

Styles, E. D., 1982 Unstable pigmenting factors. Maize Genet. Coop. News Lett. **56:** 160–161.

Styles, E. D., and O. Ceska, 1977 The genetic control of flavonoid synthesis in maize. Can. J. Genet. Cytol. **19:** 289–302.

Styles, E. D., and O. Ceska, 1989 Pericarp flavonoids in genetic strains of *Zea mays*. Maydica **34:** 227–237.

Styles, E. D., B. Erfanifar and O. Ceska, 1987 An unstable factor for orange pigment. Maize Genet. Coop. News Lett. **61:** 171.

Todd, J. J., and L. O. Vodkin, 1996 Duplications that suppress and deletions that restore expression from a chalcone synthase multigene family. Plant Cell **8:** 687–699.

Wienand, U., U. Weydemann, U. Niesbach-Klösgen, P. Peterson and H. Saedler, 1986 Molecular cloning of the c2 locus of *Zea mays*, the gene encoding chalcone synthase. Mol. Gen. Genet. **203:** 202–207.

Winkel-Shirley, B. 2001 Flavonoid biosynthesis. A color model for genetics, biochemistry, cell biology and biotechnology. Plant Physiol. **126:** 485–493.

Ye, F., and E. Singer, 1996 RIGS (repeat-induced gene silencing) in Arabidopsis is transcriptional and alters chromatin configuration. Proc. Natl. Acad. Sci. USA **93:** 10881–10886.

Zhang, P., S. Chopra and T. Peterson, 2000 A segmental gene duplication generated differentially expressed *myb*-homologous genes in maize. Plant Cell **12:** 2311–2322.

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