Paramutation, an Allelic Interaction, Is Associated With a Stable and Heritable Reduction of Transcription of the Maize *b* Regulatory Gene

Garth I. Patterson, Christopher J. Thorpe and Vicki L. Chandler

Institute of Molecular Biology, University of Oregon, Eugene, Oregon 97403

Manuscript received May 10, 1993
Accepted for publication July 17, 1993

ABSTRACT

The *b* gene of maize encodes a transcriptional activator of anthocyanin pigment biosynthetic genes. Certain *b* alleles undergo paramutation: a unidirectional, heritable alteration of one allele caused by the presence of another allele. *B-I* (intensely pigmented plant) is always changed to *B′* (weakly pigmented plant) in the *B′/B-I* heterozygote, such that all progeny receive the *B′* allele. The "new" *B′*, which was *B-I* in the previous generation, is weakly pigmented and fully capable of changing another *B-I* allele into *B′*. It was not previously known whether paramutation is associated with altered *b* expression, altered B protein function or both. Our results show that *B′* acts in trans to suppress the transcription of *B-I*, with transcription remaining low in subsequent generations, even when the original *B′* allele segregates away. The products of *B-I* and *B′* are equally capable of activating the transcription of their target genes, indicating they are functionally equivalent. Genomic restriction maps, DNA sequence and methylation of *B′* and *B-I* were compared. Despite dramatic differences in phenotype and transcription of *B′* and *B-I*, no evidence for rearrangements, changes in sequence or changes in methylation was found. These results provide no support for models involving "dominant negative" proteins, gene conversion or transposable element interactions. We suggest that *b* paramutation involves a physical interaction between the alleles that suppresses transcription and promotes a change in chromatin structure that is heritable.

PARAMUTATION is a directed, heritable alteration of one allele caused by the presence of another allele of the gene. Paramutation has been observed in several different plant systems and has been extensively studied with the maize genes *b* and *r* (COE 1966; BRINK 1973). *b* and *r* are functionally duplicate genes that encode basic-helix-loop-helix transcriptional regulators required for expression of the anthocyanin biosynthetic genes (reviewed in DOONER, ROBBINS and JORGENSON 1991). Homozygous *B-I* plants accumulate high levels of anthocyanin pigment, producing intensely purple plants. Plants homozygous for *B′*, a spontaneous mutant of *B-I*, accumulate anthocyanins in the same tissues as plants with *B-I*, but at a lower level, producing weakly pigmented plants (COE 1966, 1979). When *B′* and *B-I* are combined in a heterozygote, the phenotype is very similar to that of *B′/B′* plants; therefore, *B′* is almost completely dominant to *B-I*. The dominance of *B′* is unusual in that *B-I* is typically dominant to *b* alleles that produce low levels of pigment. Remarkably, *B-I* is never transmitted from a *B′/B-I* heterozygote; it is always changed to *B′*. For example, when a *B′/B-I* plant is crossed to a plant homozygous for the nonfunctional *b* allele, all progeny are *B′/b*. Similarly, all progeny of a self-pollination of a *B′/B-I* plant are *B′/B′*. The failure to segregate is not due to segregation distortion, as markers linked to *B-I* segregate normally. The "new" *B′*, which was *B-I* in the previous generation, produces weak pigment as does the original *B′*. Paramutation by the "new" *B′* is also 100% efficient; when the "new" *B′* allele is heterozygous with a "naive" *B-I* allele (*i.e.*, a *B-I* allele not previously exposed to *B′*), the *B-I* allele is changed to *B′*, 100% of the time. The capacity to paramutate is tightly linked to *B′*, suggesting that paramutation is an allelic interaction (COE 1966; G. I. PATTERSON and V. L. CHANDLER, unpublished data). *B-I* spontaneously changes to *B′* at a frequency higher than a typical mutation event. In our stocks with a W23 genetic background, 1–10% of the progeny of a *B-I/B-I* plant have the *B′* allele.

Paramutation also occurs at the *r* gene, but *b* and *r* paramutation have important differences in penetrance and stability (COE 1966; DOONER, ROBBINS and JORGENSON 1991). Once *B-I* changes to *B′*, the alteration is completely stable, and revertants to *B-I* are not observed (COE 1966; G. I. PATTERSON and V. L. CHANDLER, unpublished data). In contrast, *R-r′* tends to revert back to the standard *R-r* allele. In addition, the "new" *B′* is able to paramutate a naive *B-I* allele 100% of the time in a secondary paramutation event. *R-r′* alleles are only weakly paramutagenic. Other examples of paramutation in plants are cited in BRINK (1973), and these examples are also more weakly penetrant than *b* paramutation.

Several models can be proposed to account for the unusual dominance of *B'*, and for the heritable change of *B-I* to *B'* in the heterozygote. The *B'* allele might be dominant because the protein it produces interferes with the function of the *B-I* protein. Many examples of "dominant negative" proteins have been seen; for example, the dominant *C1-I* allele of the *c1* gene in maize interferes with the function of the *C1* allele (GOFF, CONE and FROMM 1991). Alternatively, the dominance could be due to protein factors bound to the *B'* allele acting in trans to suppress the transcription of the *B-I* allele. This model is favored for trans-inactivation and transvection in Drosophila (PIRROTTA 1990), and has been proposed for trans-inactivation in plants (MATZKE and MATZKE 1993) and for dominance of the allele that causes Huntington's disease (SABL and LAIRD 1992). The heritable alteration of *B-I* by *B'* can be explained by proposing that the interaction of proteins bound to *B'* with *B-I* has a heritable epigenetic effect on *B-I*. Other models for the heritable alteration of *B-I* involve changes in activity of transposable elements proposed to reside in *b*, or directed gene conversion or DNA rearrangements.

These models lead to different predictions about the expression, structure and function of *b* in *B'/B'*, *B'/B-I* and *B-I/B-I* plants. Examination of the accumulation, structure and transcription rate of *b* RNA in *B'/B'*, *B'/B-I* and *B-I/B-I* plants showed that *b* paramutation affects *b* transcription. The activation of the genes regulated by *b* was measured, and no indication of differences in activity of the products of *B-I* and *B'* was seen. The structure of the *B'* and *B-I* alleles was compared by restriction mapping and DNA sequencing, and cytosine methylation was compared by digesting genomic DNA with methylation-sensitive restriction enzymes. This analysis revealed no differences between *B-I* and *B'*; therefore, paramutation does not correlate with obvious changes in DNA structure or methylation. These results do not support models involving a "dominant negative" protein, gene conversion, or transposable element interactions. A model in which an allelic interaction promotes heritable changes in chromatin structure is suggested.

MATERIALS AND METHODS

Plant stocks: Stocks containing the *B'*, *B-I*, *b-W23*, *gl2 b⁻ wt* and *b'-v4* alleles were obtained from E. H. COE, JR. (University of Missouri, Columbia). Additional *B'* alleles were obtained from spontaneous mutations of *B-I* to *B'*. We frequently observed exceptional *B'* plants in self-pollinated progeny of *B-I* plants, as described (COE 1966). These progeny were self-pollinated to make *B'/B'* stocks and crossed to their *B-I* siblings to make *B'/B-I* stocks. The *B'* and *b'-v4* stocks obtained from E. H. COE, JR. are in a K55 genetic background. The *B-I* stock and the *B'* and *B'/B-I* stocks derived from it are in a W23 background. The experiments in Figures 1–4 used the W23 stocks, the experiments in Figure 5 used both W23 and K55 stocks. *gl2 b⁻ wt* is in a complex genetic background, and contains a *b⁻* allele different from *b-W23*, as determined by restriction mapping (data not shown). All stocks contained the *r-g:Stadler* allele of *r* and dominant alleles for the other genes involved in anthocyanin biosynthesis in plant tissues (*a1*, *a2*, *c2*, *bz1*, *bz2*, *pl*; see COE, NEUFFER and HOISINGTON 1988 for review).

DNA materials: Maize DNA was isolated from leaves (DELLAPORTA, WOOD and HICKS 1983) or from immature ears (RIVIN, ZIMMER and WALBOT 1982). The isolations of *B-I* genomic and cDNA clones and *b-W23* genomic clones were described previously (RADICELLA *et al.* 1992; CHANDLER *et al.* 1989). The *b-W23* clone was used to isolate *B'* genomic clones. The source of the *B'* allele is as follows. A *B-I* stock was crossed to a *b'-v4* stock. The *b'-v4* allele is a derivative of the mutable *B-v* allele (COE 1966) and does not activate the anthocyanin pathway; therefore, *b'-v4/b'-v4* plants are green. A *B-I/b'-v4* heterozygote was test crossed to a *gl2 b⁻ wt* stock. The *b⁻* allele is a recessive, nonfunctional *b* allele; *b⁻/b⁻* plants are green. In *b'-v4/B-I* heterozygotes, as in *B'/B-I* heterozygotes, *B-I* is changed into *B'*; therefore, in the test cross to the *gl2 b⁻ wt* stock, *B'/gl2 b⁻ wt* (light purple) and *b'-v4/gl2 b⁻ wt* (green) plants were produced. DNA isolated from *B'/(gl2 b⁻ wt)* plants was digested with restriction enzyme and size-fractionated, which allowed the DNA from the *B'* allele to be separated from the *b⁻* allele. Two *B'* restriction fragments were cloned. A 3.5-kb *Spe*I fragment spanning −1,487 bp to ~+1,600 bp (relative to the transcription start site) was cloned into lambda Long C (SHORT *et al.* 1988) and a 10.5-kb *Bcl*I fragment spanning +190 bp to ~+11,000 bp was cloned into lambda EMBL3 (FRISCHAUF *et al.* 1983). A 3.2-kb *Bcl*I fragment of the *B-I* allele spanning ~−3,000 to +190 was cloned into lambda Bv2 (NOREEN MURRAY, University of Edinburgh, Scotland). Previously cloned *B-I* sequences spanned −836 to ~+12,000 (RADICELLA *et al.* 1992). DNA sequencing was by the chain-termination method (SANGER, NICKLEN and COULSON 1977) using Sequenase (U.S. Biochemical). The region sequenced in *B-I* and *B'* clones has been submitted to EMBL (accession number X70791). The maize actin clone (pMAC1) is described in MEAGHER *et al.* (1983). The *a1* probe is a 1.5-kb *Ava*I fragment of a genomic clone (pAL; SCHWARZ-SOMMER *et al.* 1987), and the *bz1* probe is a 2.1-kb *Pst*I fragment of a genomic clone (pMBzP17; FURTEK *et al.* 1988) and the *c2* probe is a cDNA (WIENAND *et al.* 1986). The *r* probe is a subclone (CHANDLER *et al.* 1989) from an *R(S)* genomic clone (DELLAPORTA *et al.* 1988). The *LHCP* cDNA clone is described in NELSON *et al.* (1984) and the *ubiquitin* clone (pCA210) in CHRISTENSEN and QUAIL (1989).

RNA isolation and analysis: RNA was isolated from husk, sheath and tassel according to the guanidinium thiocyanate method (CHIRGWIN *et al.* 1979). Electrophoresis and transfer to nitrocellulose or MSI nylon (Fisher) were according to AUSUBEL *et al.* (1987). RNA was directly affixed to nylon or nitrocellulose with a slot blotter according to RIVIN (1986), except that the RNA was denatured with a formaldehyde/formamide buffer (AUSUBEL *et al.* 1987). Each blot includes RNA from the *gl2 b⁻ wt* genotype as a control for background hybridization, as this genotype does not make *b* RNA that can be detected in Northern blot analysis. Hybridizations were performed as described (CHANDLER *et al.* 1989). Quantitation of radioactive signal was performed on the AMBIS radioactive detection system (Ambis, Inc.). The calculation of hybridization intensity for a particular sample was performed by measuring the intensity of hybridization to the probe of interest, subtracting the value for intensity of hybridization to the *b⁻* control sample, and dividing by intensity of hybridization to *actin* (as a control for loading differences).

***In vitro* transcription assays:** To obtain nuclei and steady state RNA from the same samples, husk tissue was homogenized in a blender, an aliquot of the liquid from the blended material was removed and steady-state RNA isolated by the guanidinium thiocyanate method (CHIRGWIN *et al.* 1979). Nuclei were isolated from the same liquid as described (WATSON and THOMPSON 1986). ATP, GTP, CTP and ^{32}P labeled UTP were added to ~5 × 10^6 nuclei in each sample, and transcription reactions and transcript isolation were performed as described in AUSUBEL *et al.* (1987). The radioactive RNA was hybridized to 5 μg of linear, denatured DNA that had been affixed to nitrocellulose by slot blotting. Hybridization conditions were the same as those used for Northern blots. The amount of hybridization was measured by quantitation of radioactive signal using the AMBIS radioactive detection system (Ambis, Inc.). Hybridization to maize *ubiquitin* and *LHCP* were used to control for differences in the efficiency of the transcription reaction from sample to sample, and hybridization to the plasmid vector ptz19 was used as a control for background hybridization. The calculation of hybridization intensity for a particular sample was performed by measuring the intensity of hybridization of labeled RNA to the DNA of interest, subtracting the value for intensity of hybridization to the ptz19 control DNA, and dividing by intensity of hybridization to *ubiquitin*.

DNA gel blot analysis: DNA gel blot analysis and hybridization were performed as described (CHANDLER, TALBERT and RAYMOND 1988), except that MSI nylon (Fisher) was used. All DNA digests were done with a 5- to 10-fold excess of enzyme according to manufacturer's specifications. Probes were labeled using random hexamer priming (FEINBERG and VOGELSTEIN 1983). Quantitation of hybridization was performed by laser densitometry (Biorad Instruments Model SL-504-XL).

Methylation analysis: Sites tested for extent of cutting with methylation-sensitive enzymes are listed as follows: (site number; location; enzyme; *B′/B′*, *B′/B-I*, *B-I/B-I*). For example, the first listing is for site 1 in Figure 5, which is a *Hha*I site ~−9000 bp from the transcription start site. The digestion of this site was tested in DNA samples from four *B′/B′* plants, two *B′/B-I* plants, and four *B-I/B-I* plants. Sites tested: (1; ~−9000; *Hha*I; 4, 2, 4), (2; ~−7500; *Ava*I; 1, 0, 1), (3; ~−7500; *Sal*I; 1, 0, 1), (4; ~−6500; *Eag*I; 2, 2, 2), (5; ~−4200; *Nae*I; 1, 1, 1), (6; ~−3500; *Pvu*II; 2, 0, 1), (7; ~−3000; *Hpa*II and *Msp*I; 4, 2, 3), (8; ~−2400; *Hpa*II and *Msp*I; 4, 2, 3); (9; ~−2400; *Xho*I; 2, 0, 1), (10; ~−2300; *Ava*II; 5, 3, 4), (11; ~−1800; *Hpa*II and *Msp*I; 4, 2, 3), (12; ~−1500; *Ava*II; 5, 3, 4), (13; −1282; *Dde*I; 6, 6, 6), (14; −1259; *Ava*I and *Xho*I; 5, 2, 4), (15; −1098; *Alu*I; see Table 2), (16; −1036; *Tha*I; 1, 0, 1); (17; −522; *Sal*I; 1,0, 1); (18; −495; *Sau*96I; 5, 3, 4), (19; −214; *Hae*III; 7, 6, 8), (20; −191; *Sau*3A; 4, 3, 4), (21; −188; *Pvu*I; 2, 0, 1), (22; −133; *Hpa*II and *Msp*I; 4, 2, 3), (23; −8; *Dde*I; 2, 0, 1), (24; 227; *Hinf*I; 2, 0, 1), (25; 318; *Nhe*I; 2, 0, 1), (26; 461; *Tha*I; 1, 0, 1), (27; 461; *Hha*I; 6, 2, 5), (28; 474; *Dde*I; 2, 0, 1), (29; 484; *Hpa*II and *Msp*I; 4, 2, 3), (30; 506; *Pst*I; 2, 0, 1), (31; 519; *Hae*III; 7, 6, 8), (32; 547; *Tha*I; 1, 0, 1), (33; 581; *Ava*II; 5, 3, 4), (34; 720; *Bam*HI; 1, 0, 1), (35; 783; *Hinf*I; 2, 0, 1), (36; 823; *Ava*I; 3, 2, 5), (37; 853; *Tha*I; 1, 0, 1), (38; 854; *Hha*I; 2, 0, 1), (39; 877; *Hae*III; 1, 0, 1), (40; 878; *Hpa*II and *Msp*I; 4, 2, 3), (41; 878; *Nae*I; 2, 0, 1), (42; 889; *Dde*I; 2, 0, 1), (43; 941; *Bgl*I; 1, 0, 1).

The general strategy used to examine cutting of *b* with methylation sensitive enzymes was to digest with two restriction enzymes. One enzyme was a methylation-insensitive enzyme, used to produce a fragment of known size independent of methylation state. The second enzyme was a methylation-sensitive enzyme. Cutting by the methylation-sensitive enzyme was monitored on Southern blots by the production of smaller fragments from the fragment of known size. This strategy was used for sites 6–41 in Figure 5B. A second strategy, used for sites 1–5 in Figure 5A, was to digest solely with a methylation-sensitive enzyme. In these cases, a single band was observed in all three genotypes, demonstrating that the enzyme cut to completion.

For sites 6–18 (Figure 5B), several lines of evidence suggest that the failure of the enzymes to cut was due to cytosine methylation, rather than some other cause. First, we know that the enzymes were working efficiently in each case because there was always at least one site in each digest that was cut to completion. Second, site 14 (Figure 5B) was cut to completion by the methylation insensitive isoschizomer *Taq*I. Finally, in general, the enzymes failed to cut only at sites that contain CpG or CpNpG, the canonical methylated sites in maize. The one exception was an *Hae*III site at −493 relative to the transcription start site. *Hae*III is sensitive to methylation at a C residue in the recognition site, but the C residue in this site is not in the context of a CpG or CpNpG. This site was only partially digested in all samples tested. The enzyme was functioning efficiently, as demonstrated by the fact that other *Hae*III sites in the vicinity were cut to completion (sites 19, 31, 39, Figure 5B). Either some noncanonical methylation was present, or there was another reason for the failure to cut to completion. For example, the enzyme may have sensitivity to methylation of cytosine residues flanking the recognition site (MCCLELLAND and NELSON 1992). A similar example of a methylation-sensitive enzyme failing to cut at a restriction site with no CpG or CpNpG sequences has been observed (WALBOT 1992).

We examined the sequenced region for CpG content by two standard methods. The first compares the observed frequency of the CpG dinucleotide with the frequency expected from C and G content (GARDINER-GARDEN and FROMMER 1992). The second compares CpG content with GpC content (ANTEQUERA and BIRD 1988). These two methods gave similar results, and the second method was used to generate the graph in Figure 5C.

RESULTS

Paramutation is associated with low *b* RNA levels but not with changes in function of the B protein: The phenotypes of *B-I/B-I*, *B′/B-I* and *B′/B′* plants are shown in Figure 1. In husks, leaf sheaths, culms and tassels (Figure 1, A–C), homozygous *B-I* plants have intense pigmentation. Homozygous *B′* plants have weak pigmentation in the tissues shown (Figure 1, A–C), and have less pigment than *B-I/B-I* in every tissue (COE 1979). When *B-I* is heterozygous with most *b* alleles, it is dominant, producing intense pigment. However, when *B-I* is heterozygous with *B′*, pigmentation is strikingly reduced (Figure 1, A–C). The *B′/B-I* phenotype is similar to that of *B′/B′* but, on average, *B′/B-I* plants are just slightly darker than *B′/B′*.

To determine whether the low pigmentation in *B′/B′* and *B′/B-I* plants is correlated with altered expression of the *b* gene, the amount and structure of *b* RNA was examined. Northern blots were performed using total RNA isolated from several different tissues (Figure 2). *b* RNA levels correlated with pigment levels and were lower in husks, tassels and leaf sheaths

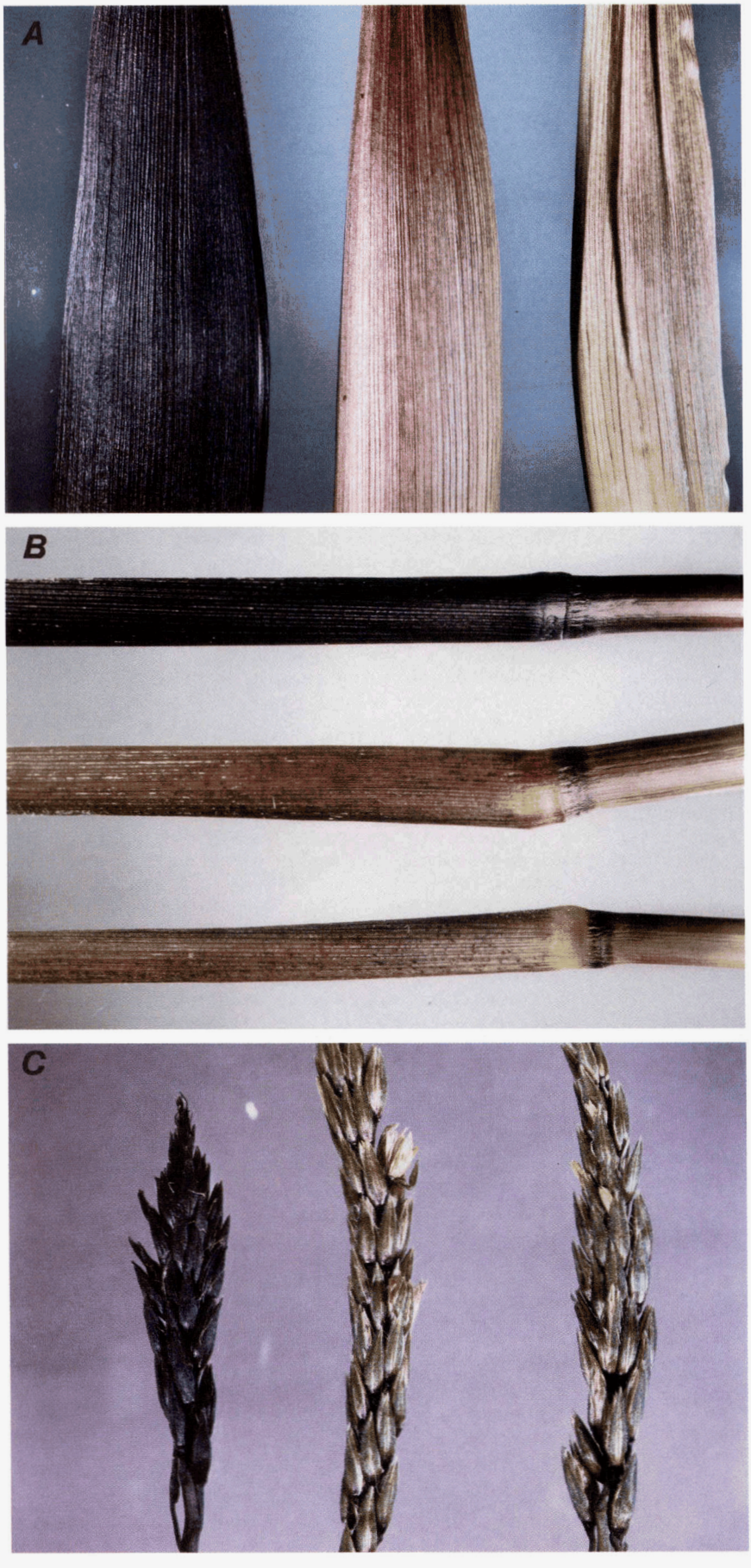

FIGURE 1.—Phenotypes of *B-I/B-I*, *B′/B-I* and *B′/B′*. All material was collected at flowering from plants in a W23 genetic background. (A) Female inflorescence (ear). The husk shown is the fourth innermost. (left-right: *B-I/B-I*, *B′/B-I* and *B′/B′*). (B) Leaf sheath and culm (stem) (top-bottom: *B-I/B-I*, *B′/B-I* and *B′/B′*). (C) Branch of tassel (male inflorescence) (left-right: *B-I/B-I*, *B′/B-I* and *B′/B′*).

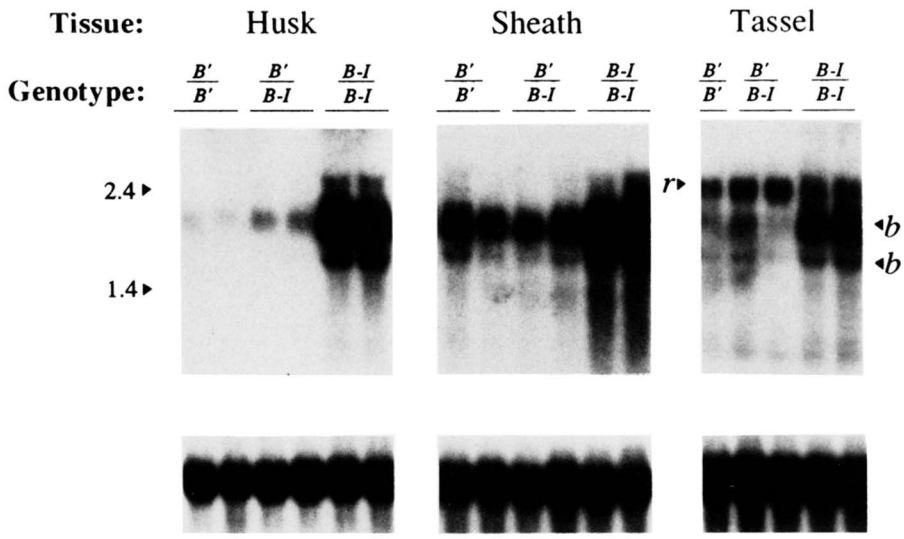

FIGURE 2.—Blot analysis of total RNA isolated from *B′/B′*, *B′/B-I* and *B-I/B-I* plants. Ten μg of total RNA isolated from husk, sheath or tassel material of mature plants were electrophoresed in agarose gels, transferred to nylon membrane and probed with a *B-I* cDNA probe (top). Molecular weight of size markers in kb is indicated. In the lanes with RNA from tassel, the bands are marked as "*r*" or "*b*" as described in text. To demonstrate equal loading in each lane, the blot was stripped of *b* probe and rehybridized with a maize actin clone (bottom).

from *B′/B′* and *B′/B-I* plants than from *B-I/B-I* plants. In all three genotypes, three bands hybridized well to the *b* probe in husk and sheath tissue (Figure 2). All of these RNAs showed the same regulation, in that all were present at higher levels in tissues from *B-I/B-I* plants than in tissues from *B′/B-I* or *B′/B′* plants. The middle, most prominent band is the same size as the RNA previously cloned as a cDNA from *B-I* (RADICELLA *et al.* 1992). The other bands may represent unprocessed or differently spliced products of the *b* gene, as seen previously (RADICELLA *et al.* 1992). The two faint bands in the lanes of RNA from *B-I/B-I* plants are also seen in the RNA from *B′/B-I* and *B′/B′* plants upon longer exposure (data not shown). These results show that the *b* transcripts are similar in size in all three genotypes.

In the tassel, two bands showed the same regulation that was observed in husk and sheath; *B-I/B-I* tassel had more *b* RNA than *B′/B-I* or *B′/B′* (marked "*b*," Figure 2). An additional band was present at the same intensity in all three genotypes. The *b* gene is homologous to the *r* gene in maize (RADICELLA, TURKS and CHANDLER 1991); to determine whether the band represented RNA from the *r* gene, the blot was stripped and reprobed with an *r* probe. The band hybridized more strongly to the *r* probe than to the *b* probe (data not shown), and is marked "*r*" in Figure 2. The *r* RNA shown in Figure 2 was not affected by *b* paramutation, which is consistent with genetic tests that indicate *b* is unaffected by *r* paramutation (COE, NEUFFER and HOISINGTON 1988). The two RNAs that were produced by *b* in the tassel are regulated similarly to those produced by *b* in husk and sheath; the RNAs in each genotype were similar in size but were less abundant in *B′/B′* and *B′/B-I* plants than in *B-I/B-I* plants. These results demonstrate that, in a *B′/B-I* heterozygote, the expression of *B-I* is suppressed by *B′*. Since the heterozygote transmits only *B′*, the expression of *B-I* is heritably altered by *B′*.

These initial experiments indicated that *B′* is regulated differently from *B-I*, but could not detect small differences in *b* RNA structure. The 5′ end of the *B-I* transcript has been characterized previously by cDNA cloning and sequencing, and by RNAse and S1 protection assays (RADICELLA *et al.* 1992). To determine whether paramutation affects the structure of the 5′ end of the transcript, RNA isolated from *B′/B′*, *B′/B-I* and *B-I/B-I* plants was compared using RNAse protection. A labeled antisense RNA probe spanning genomic sequences from −188 to +506 bp relative to the transcription start site in *B-I* (RADICELLA *et al.* 1992) protected an ~100-nt species of RNA from each of the three genotypes (data not shown). This corresponds well to the 99-nt exon 1 previously characterized for *B-I* (RADICELLA *et al.* 1992). Previous experiments have shown that the splicing event that fuses exon 1 to exon 2 is not efficient in *B-I*, resulting in a small amount of RNA that retains intron 1 (RADICELLA *et al.* 1992). This intron adds ~300 nucleotides to the untranslated leader region of the RNA, which might affect mRNA stability or translation efficiency. To test whether differences in splicing of this intron might account for differences in phenotype between *B′* and *B-I*, an additional RNAse protection experiment was done. An antisense RNA probe of a cDNA clone that had exon 1 of *B-I* fused to exon 2 (RADICELLA *et al.* 1992) protected similarly sized fragments in all three genotypes, and the proportion of the bands representing spliced message to bands representing unspliced message was similar in all genotypes (data not shown). The pattern of bands produced in these RNAse protection assays is similar in all three genotypes; there is no indication of differences in RNA structure.

Results of the RNAse protection and Northern blot analyses suggested that paramutation affects the level,

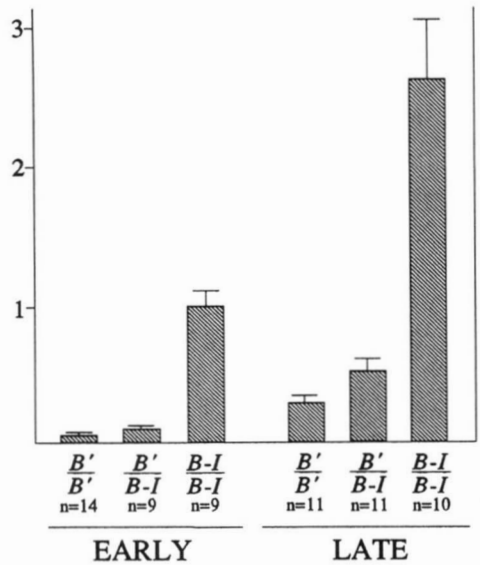

FIGURE 3.—Quantitation of RNA. Relative RNA amounts were measured in leaf sheaths of the three genotypes indicated. The *n* values represent the number of different plants of each genotype that were tested. Ten μg of each RNA sample were loaded in quadruplicate on a slot blot apparatus. The values indicated by the bars are an average of all loadings of all samples for each genotype. All values are normalized to the value for *B-I/B-I* at the early stage. Error bars show the standard error. The sheaths at the late stage in this figure are equivalent in age to the sheaths in Figures 1 and 2.

rather than the structure, of *b* RNA. To quantify this effect, total RNA isolated from sheath tissue of *B'/B'*, *B'/B-I* and *B-I/B-I* plants was examined using slot blot analysis. In the sheath, hybridization to the *b* probe reflects *b* RNA level only, as no *r* RNA was seen on Northern blots (Figure 2). RNA was isolated from sheaths at two stages. The first stage was when the sheath had nearly finished growing, and the second was 10 days later. At the first stage, sheaths from *B-I/B-I* plants had substantial pigment, whereas pigment in sheaths from *B'/B-I* or *B'/B'* plants was very faint. The amount of *b* RNA in a *B-I/B-I* homozygote was ~20-fold higher than in *B'/B'* and ~10-fold higher than in *B'/B-I* (Figure 3). On average, *B'/B-I* heterozygotes had ~twofold more *b* RNA than *B'/B'*. At the second stage from which RNA was isolated, pigment in sheaths of *B-I/B-I* plants was very intense, and pigment in sheaths of *B'/B-I* and *B'/B'* plants was moderate (Figure 1B). In sheaths at this time, as at the previous time, *b* RNA levels were highest in *B-I/B-I* (Figure 3). At this later time, *b* RNA in *B-I/B-I* sheaths was ~fivefold higher than in *B'/B-I* and ~10-fold higher than in *B'/B'*.

The transcription of the target genes *a1* and *bz1* is dependent on *b* function (GOFF *et al.* 1990). If the *B'* and *B-I* RNAs are translated with equivalent efficiency, and the *B'* and *B-I* proteins are equally able to activate transcription, the ratio of *b* RNA to *a1* and *bz1* RNA should be approximately the same in all three genotypes. If the product of the *B'* allele is partially defective in activating transcription, we would expect that the ratio of *b* RNA to *a1* and *bz1* RNA would be higher in *B'/B'* than in *B-I/B-I* plants. Hybridization to probes from *a1*, *bz1* and *c2* (another target gene) was measured in the same RNA samples that were used to generate Figure 3. On average, the ratio of *b* RNA to biosynthetic gene RNA was similar in *B'/B'* and *B-I/B-I* samples (Table 1), indicating that the products of *B'* and *B-I* are equally able to activate the transcription of the target genes. The *c2* probe used recognizes both the *c2* gene and the homologous *whp* gene (FRANKEN *et al.* 1991). The RNA detected on slot blots may include some *whp* RNA in addition to *c2* RNA. In these experiments, the ratio of *b* RNA to *a1* RNA was ~2.5-fold higher in *B'/B-I* than in the other two genotypes. Given the variation from experiment to experiment, it is unclear whether this difference is significant.

TABLE 1

Ratio of *b* RNA levels to anthocyanin biosynthetic gene RNA levels

Ratio	Genotype		
	B'/B'	*B'/B-I*	*B-I/B-I*
b/bz1	0.71 ± 0.08[a]	1.20 ± 0.29	1
b/a1	1.20 ± 0.36	2.47 ± 0.61	1
b/c2[b]	0.75 ± 0.16	1.32 ± 0.10	1

[a] Numbers represent ratio of *b* RNA to anthocyanin biosynthetic gene RNA in leaf sheath; all values are normalized to *B-I/B-I*. The normalized values for the early and late stages were similar, and the numbers shown represent an average of all samples.

[b] The c2 probe used recognizes both *c2* and *whp*, which are homologous genes encoding chalcone synthease (FRANKEN *et al.* 1991).

Paramutation affects *b* transcription: The experiments described above demonstrate that the decrease in pigmentation caused by *b* paramutation correlates with a decrease in *b* RNA levels. Differences in *b* RNA accumulation could be caused by differences in transcription, RNA stability or both. To distinguish between these possibilities, relative transcription rates were measured in nuclei isolated from *B'/B'*, *B'/B-I* and *B-I/B-I* husk tissue and steady-state RNA levels were measured in the same samples. This procedure does not measure RNA stability directly, but differences in *b* mRNA stability in the three genotypes tested should result in a lack of correlation between steady-state levels of *b* RNA and the relative transcription rates of *b*.

Transcription of *b* was relatively high in nuclei from *B-I/B-I* husks, and low in nuclei from *B'/B-I* and *B'/B'* husks (Figure 4A). The average relative rate of *b* transcription was 10-fold higher in *B-I/B-I* husk than in *B'/B-I* or *B'/B'* (Figure 4B), and the steady-state levels of RNA were well-correlated (Figure 4C). Small

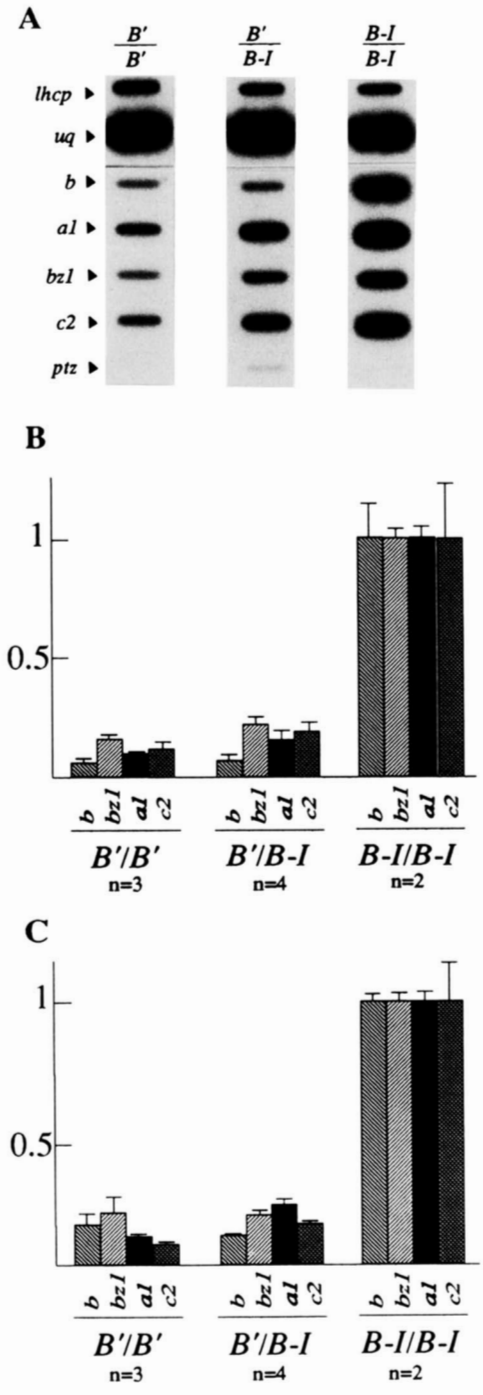

FIGURE 4.—Transcription assays in nuclei isolated from husks. Nuclei were isolated from husks of *B'/B'*, *B'/B-I* and *B-I/B-I* plants, labeled rNTPs were added to the nuclei, and transcription allowed to continue. The resultant RNA was hybridized to denatured plasmids containing clones of maize genes, as indicated. (A) One example of the hybridization signal produced by each genotype is shown. *LHCP* and *Uq* are maize genes that control for efficiency of the transcription assay, and ptz plasmid DNA with no maize insert is included as a control for background hybridization. (B) A quantitation of the hybridization signal in the samples shown in panel A and other samples from the same experiment. The *n* values indicate the number of husk samples that were used to generate the average for each genotype. The error bars show the standard error. Three other experiments produced qualitatively similar results, but were not quantified (not shown). (C) Level of steady-state RNA. Steady-state RNA was isolated from the same husk samples as in panel B, and relative levels were measured by slot blot analysis as described in Figure 3.

differences cannot be ruled out, but there is no evidence for changes in RNA stability. Clearly, the decrease in *b* RNA accumulation due to paramutation is mostly or entirely caused by a decrease in transcription.

The rates of transcription of *bz1* and *a1* correlated with the rate of *b* transcription (Figures 4, A and B), as expected, since *b* is known to induce transcription from *bz1* and *a1* promoters in transient transformation assays (GOFF *et al.* 1990). The ratio of *b* to *c2* homologous RNA is similar in the three genotypes (Figure 4). Thus, the production of these RNAs is regulated by *b* at the level of transcription. The transcription rate of control genes (*ubiquitin* and *LHCP*) was not affected by *b* genotype (Figure 4A).

No alterations in DNA structure or sequence were detected between *B'* and *B-I*: A simple model to explain the stable and heritable change in transcription that occurs when *B-I* is paramutated is that a rearrangement, duplication or amplification of DNA sequences occurs within or near the *b* gene. Accordingly, detailed restriction maps of the *B-I* and *B'* alleles were generated using Southern blots of genomic DNA. For every site tested, DNA from plants with *B'* or *B-I* alleles was digested and compared on adjacent lanes of a gel. In no case was a band present in DNA from one genotype and absent in the other, demonstrating that all sites tested were the same in *B-I* and *B'*. Five sites were mapped in the region between −6,000 and −11,000 (all distances are in base pairs, and are relative to the transcription start site), and three sites were mapped in the region from +6,000 to +13,000 (Figure 5A). Over 100 sites were mapped in the region from −6,000 to +6,000. Only a subset of these sites are shown in Figure 5A and Figure 5B. The structure of the *B'* and *B-I* alleles are identical at this level of resolution; the transcribed region and surrounding sequences of *b* are not detectably rearranged, duplicated or amplified. A small rearrangement, or a rearrangement outside this 24-kb region cannot be eliminated. Since paramutation affects *b* transcription, *B'* and *B-I* clones that included ~2,200 bp of sequence surrounding the transcription start site were sequenced. The region sequenced is shown as a line below the map in Figure 5B. The sequences of the *B-I* and *B'* clones were identical in this region.

Analysis of the sequence of *B-I* and *B'* revealed that these alleles contain a CpG island like those that have been previously identified in animals (BIRD 1986) and plants (GARDINER-GARDEN and FROMMER 1992). In most sequenced regions of the maize genome, the

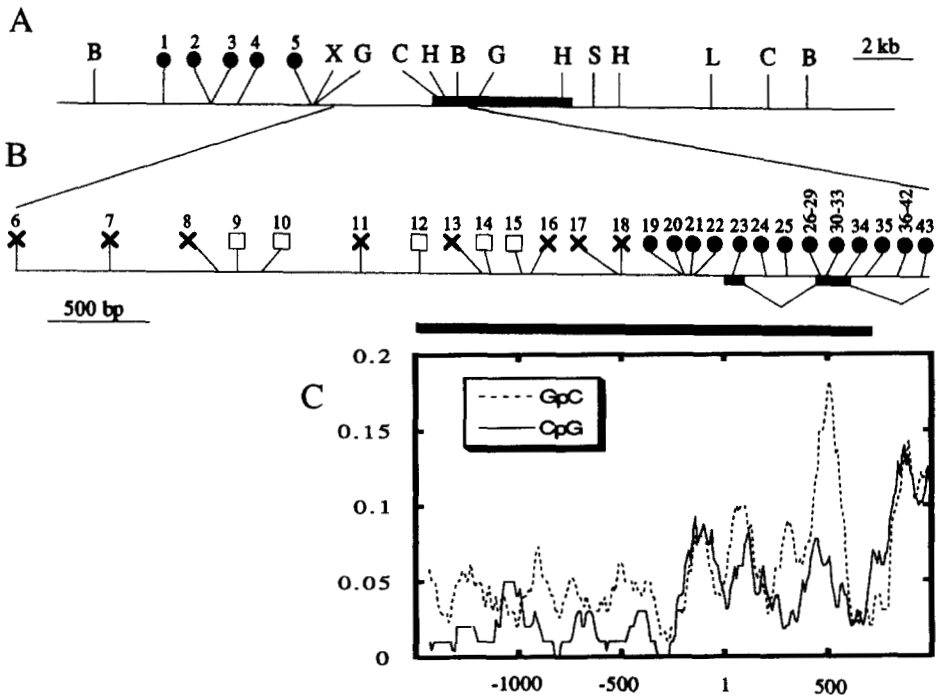

FIGURE 5.—Restriction enzyme and DNA sequence analysis of *B-I* and *B′*. (A) Restriction map of *B-I* and *B′*. Both alleles are represented by one map, as no differences in cutting by restriction enzymes are seen. The bold line represents the 4-kb region transcribed to produce the *B′* and *B-I* RNA. B, *Bam*HI; C, *Bcl*I; G, *Bgl*II; H, *Hind*III; L, *Bgl*I; S, *Sal*I. Numbered sites are described in (B). Not all restriction sites cut by a particular enzyme are shown on the map. (B) Detailed map of the region surrounding the transcription start site. Again, both alleles are represented by one map. Sites that can be blocked by cytosine methylation are numbered; the restriction enzyme that corresponds to each site, and the number of DNA samples tested is listed in MATERIALS AND METHODS. An X represents a site that is cut in less than 10% of the DNA in an average digest by a particular methylation-sensitive enzyme. An open box represents a site that is cut in 10–50% of the DNA in an average digest by a methylation-sensitive enzyme. A closed circle represents a site that is cut to completion in digests by a methylation-sensitive enzyme. The first two exons of the alleles as mapped by RNAse protection and cDNA sequencing (RADICELLA *et al.* 1992) are shown on the map as bold lines. The region sequenced in both *B′* and *B-I* is shown as a line below the map. (C) A graph of CpG and GpC content. The CpG islands are regions in which CpG content is approximately equal to GpC content. Each point represents the frequency of the dinucleotide over a 100-bp window of sequence. The graph shown is collinear with the map shown in panel B.

frequency of the dinucleotide CpG is less than is expected from C and G content; CpG islands have the expected CpG content (GARDINER-GARDEN, SVED and FROMMER 1992). These same regions are relatively low in methylation (NICK *et al.* 1986; ANTEQUERA and BIRD 1988; MARTINEZ, MARTIN and CERFF 1989; BANKS, MASSON and FEDOROFF 1988; KUNZE, STARLINGER and SCHWARTZ 1988; WALBOT and WARREN 1990; LANGDALE, TAYLOR and NELSON 1991). The function of CpG islands is unclear, but in animals, it is thought that CpG islands represent areas of the genome that are in a relatively open chromatin conformation (TAZI and BIRD 1990). The CpG islands in *B-I* are from ~−200 to ~+1, and from ~+600 to ~+1000 (Figure 5C). Thus, *B′* and *B-I* have CpG islands near the transcription start site, as do other genes in maize (ANTEQUERA and BIRD 1988; LANGDALE, TAYLOR and NELSON 1991; MARTINEZ, MARTIN and CERFF 1989; BANKS, MASSON and FEDOROFF 1988; KUNZE, STARLINGER and SCHWARTZ 1988).

No differences in DNA methylation were detected between *B-I* and *B′*: Methylation of cytosine residues at the sequence CpG and CpNpG is common in maize, and methylation of cytosines correlates with some examples of non-Mendelian inheritance, such as imprinting (reviewed in SASAKI, ALLEN and SURANI 1993). Changes in methylation in CpG island sequences of maize transposable elements correlates with heritable changes in activity (KUNZE, STARLINGER and SCHWARTZ 1988; BANKS, MASSON and FEDOROFF 1988), and changes in methylation of CpG islands correlates with inactivation of genes on the *X*-chromosome of eutherian mammals (LOCK, TAKAGI and MARTIN 1987). Most significantly, the cutting of methylation-sensitive restriction sites changes substantially when paramutation occurs in an *r* allele (M. ALLEMAN and J. KERMICLE, unpublished data) and during a transgene inactivation event in petunia (MEYER, HEIDMANN and NIEDENHOFF 1993).

The cutting of *B′* and *B-I* genomic DNA by methylation-sensitive and insensitive restriction enzymes was tested. In these experiments, DNA samples from leaves or immature cobs of *B′/B′*, *B′/B-I* and *B-I/B-I* plants were cut with a 5- to 10-fold excess of restriction enzymes, transferred to membrane and examined with various probes spanning the transcribed region

TABLE 2

Cutting by *AluI*

Experiment	Genotype		
	B'/B'	*B'/B-I*	*B-I/B-I*
1	0.61 ± 0.11 (5)[a]	ND	0.05 ± 0.01 (2)
2	0.47 ± 0.11 (5)	0.37 ± 0.10 (4)	0.10 ± 0.06 (8)
3	0.29 ± 0.09 (9)	0.25 ± 0.08 (7)	0.17 ± 0.20 (5)
4	0.34 ± 0.12 (5)	0.32 ± 0.15 (6)	0.38 ± 0.20 (5)

[a] Cutting by *AluI* results in two bands. A 250-bp represents cutting at site 15 (Figure 5B), and a 450-bp band represents lack of cutting at site 15. The numbers presented were calculated as follows: (density of 250-bp band on autoradiograph)/(density of (250-bp band + 450-bp band)). The standard error is shown after each mean. The number of individuals examined is shown in parentheses.

of the gene and probes from ~2 kb upstream and downstream. Sites are considered to be indicators of methylation according to the following criteria: 1) the enzyme used is sensitive to cytosine methylation (McCLELLAND and NELSON 1992), and 2) the site tested in *B'* and *B-I* contains a cytosine residue in the context CpG or CpNpG, which are considered the canonical methylation sites in maize (GRUENBAUM *et al.* 1981). These sites are numbered in Figure 5, A and B.

In every case, when cutting of a site was not blocked by canonical methylation, the banding pattern on Southern blots was identical for all three genotypes. When the site had the potential to be blocked by methylation, the pattern of bands was also the same in all three genotypes, with one exception. Site 15 shows a weak, and perhaps not significant, correlation with genotype, as shown in Table 2. In some experiments, cutting at this site was more complete in DNA from *B'/B'* and *B'/B-I* plants than from *B-I/B-I*. In others, this site was cut to the same extent in each genotype. Sites within and between the CpG islands were cut to completion, whereas sites 5′ to the islands were poorly cut or not cut at all (Figure 5, B and C); however, the cutting was the same in DNA from *B'/B'*, *B'/B-I* and *B-I/B-I* plants.

The map does not show many sites downstream of +1000, because probes from this region cross-hybridize to the homologous *r* gene, making these sites difficult to map precisely. However, *b* probes that hybridized to the region from +1000 to +5500 were used on blots in which more than 10 different methylation-sensitive restriction enzymes were used to cut DNA from *B'/B'* and *B-I/B-I* plants. In all cases, the pattern of bands was the same in the two genotypes.

DISCUSSION

In *b* paramutation, *B-I* changes into *B'*, as indicated by a substantial decrease in anthocyanin pigment, and by the ability of the new *B'* allele to change a naive *B-I* allele into *B'*. In the experiments presented here, the change from *B-I* to *B'* was accompanied by a decrease in *b* RNA accumulation, but no change was observed in the tissue-specific pattern of accumulation, nor were the size and 5′ end of *b* RNA changed. *b* transcription was lower in *B'/B'* and *B'/B-I* husks by about 10-fold, enough to account for the difference in RNA levels. The activity of the products of the *B'* and *B-I* alleles was not different, as measured by their ability to activate the transcription of the target genes *bz1*, *a1* and *c2*. Paramutation affects the transcription, rather than the function of *b*, indicating that the light pigment of a *B'/B-I* heterozygote is not due to the *B'* protein interfering with the ability of the *B-I* protein to activate transcription of the anthocyanin biosynthetic genes. The change in *b* transcription was not associated with DNA rearrangements from −11,000 to +13,000 (relative to the transcription start at +1), as measured by the patterns of bands seen on Southern blots, nor was the DNA sequence from −1,487 to +730 altered. The *r* gene, which can also paramutate, has a complex structure, and contains duplicated sequences (ROBBINS *et al.* 1991). Hybridization with probes spanning −1,487 to +6,000 give no indication that *B'* and *B-I* contain any duplicated sequence, except that the coding region is homologous to *r*, and a 127-bp transposable element is found upstream of the coding region (described below). Cutting of *B'* and *B-I* with a large number of methylation-sensitive restriction enzymes showed that the methylation pattern between the two alleles was indistinguishable.

This technique can only measure methylation at cytosine residues that are within a restriction site; thus, the possibility of changes in methylation in other cytosines cannot be eliminated. However, the tests performed were exhaustive. In the sequenced region, from −1487 to +730, there are 120 canonical methylation sites, and the restriction enzymes used in these experiments tested methylation of 25 of these sites. The same types of enzyme sites were tested throughout the alleles; therefore, we estimate that ~20% of the methylatable cytosines from ~−3500 to ~+5500 were tested for differences in methylation, and all sites cut to the same extent. When gene expression in maize has been correlated with changes in methylation, the change in methylation has been detected by examining a smaller number of sites (LANGDALE, TAYLOR and NELSON 1991; BANKS, MASSON and FEDOROFF 1988; CHOMET, WESSLER and DELLAPORTA 1987; KUNZE, STARLINGER and SCHWARTZ 1988). When *R-r* and paramutant *R-r'* alleles (M. ALLEMAN and J. KERMICLE, unpublished data), and normal and inactivated transgenes in petunia (MEYER, HEIDMANN and NIEDENHOFF 1993) were compared, several enzymes were tested. In the case of the petunia transgene, every enzyme tested identified a site that was less cut,

and therefore, more methylated in the inactivated allele. In the case of *r*, most enzymes identified a hypermethylated site in the paramutant allele. In contrast, not one of the 22 enzymes used to cut *B-I* and *B′* identified a site that was more methylated in *B′*. The extensive change in methylation state that is correlated with heritable transgene inactivation in petunia, and with *r* paramutation, does not occur at *b*. The possibility that *b* paramutation is associated with more subtle changes in DNA methylation will be addressed by genomic sequencing when the functionally important regions of the *B′* and *B-I* promoters are identified.

Comparison of *b* paramutation and trans-inactivation: A number of interactions between endogenous genes and transgenes that share homologous sequences have been reported in plants (reviewed in MATZKE and MATZKE 1993). One feature these phenomena have in common is that, in every case tested, suppression decreases RNA accumulation. The heritability of trans-inactivation in plants is variable. In three cases tested, no heritability is seen; when the genes segregate at meiosis, the formerly suppressed gene returns to a fully active state (BOLLMAN, CARPENTER and COEN 1991; DE CARVALHO *et al.* 1992; BRUSSLAN *et al.* 1993). In two cases, heritability is seen, but the effect is weak. Only a fraction of the progeny have the gene still in the suppressed state, and even in these progeny the expression of the gene increases to some extent (GORING, THOMSON and ROTHSTEIN 1991; MATZKE and MATZKE 1991). The case of trans-inactivation of *a1* transgenes in petunia is like *b* paramutation in that suppression is meiotically heritable; all of the progeny receive the gene in the suppressed state (MEYER, HEIDMANN and NIEDENHOFF 1993).

A proposed model for trans-inactivation suggests that the homologous sequences interact by pairing (MATZKE and MATZKE 1993), and that this interaction affects the transcription of the alleles. In most cases of trans-inactivation in plants, it is not known whether transcription or RNA stability is affected. In two cases (DE CARVALHO *et al.* 1992; MOL, VAN BLOKLAND and KOOTER 1991), suppression is not accompanied by a decrease in transcription rates in isolated nuclei, suggesting that in these cases trans-inactivation is likely to be the result of post-transcriptional events. In one case, the trans-gene produced antisense RNA that was homologous to the suppressed gene (MOL, VAN BLOKLAND and KOOTER 1991), and antisense RNA is known to decrease RNA accumulation in transgenic plants (ROTHSTEIN *et al.* 1987). Two examples of trans-inactivation are similar to *b* paramutation in that the suppression of gene expression is at the level of transcription (BRUSSLAN *et al.* 1993; MEYER, HEIDMANN, and NIEDENHOFF 1993).

Models for *b* paramutation: The stability of *B′* suggests a model in which the change from *B-I* to *B′* involves a change in DNA sequence. As part of an effort to isolate mutants of *B′*, we scored over 20,000 progeny of *B′/B′* plants and have seen no revertants to *B-I*. COE (1966) made an effort to select for full or partial revertants of *B′* by selecting dark individuals from *B′/B′* and *B′/B-I* families over three plant generations. No indication of reversion was seen. Because of the stability of *B′*, COE (1966) suggested a model in which a sequence element was transferred from *B′* to *B-I* as part of paramutation. Our mapping and sequencing data show that the sequence of *B′* and *B-I* is identical in the region around the transcription start, and that no large insertions, deletions or rearrangements are present within an ~24-kb region. Paramutation is not associated with an elevated frequency of crossing over of flanking markers (COE 1966). If unidirectional gene conversion is to account for paramutation, the event would have to occur without associated crossing over, and occur 100% of the time.

COE (1966) showed that paramutation was not associated with a replacement of the *B-I* allele by *B′*. The *b′-v* allele can be distinguished from *B′* by the fact that *b′-v* produces no pigment. When *b′-v* is used to paramutate *B-I*, *B-I* changes to *B′*, not to *b′-v*. Also, *B-I* can change to *B′* spontaneously in a *B-I/B-I* plant; therefore, if a change in DNA sequence transferred by gene conversion is responsible for paramutation, the change must occur spontaneously at a relatively high rate (1–10%). There are examples of unidirectional gene conversion events that are nearly as regular as paramutation, such as the transfer of infectious intervening sequences (GIMBLE and THORNER 1992). Yeast cells switch mating types by gene conversion without associated crossing over, and this event occurs very efficiently (HABER 1983). We do not see polymorphisms in restriction maps or in the sequenced region of *B-I* and *B′*, but the sequence transferred could be smaller than we can detect by restriction mapping, or could be more distant from the locus than the restriction sites examined. Generating mutants of *B′* that are defective in the capacity to suppress or paramutate *B-I* might identify regions in which a sequence change would lie, and efforts to isolate such mutants are ongoing.

Models involving transposable elements have been proposed for *b* paramutation (D. Schwarz, personal communication, *Maize Genetics Coop. Newsl.* **63:** 42 1989) and *nivea* paramutation in Antirrhinum (KREBBERS *et al.* 1987). The region of *B′* and *B-I* from −1500 to +5000 contains only one sequence that is a repeated sequence within the maize genome; the rest of the region, when used as a probe, recognizes only *b* and homologous *r* gene sequences, and therefore probably does not contain a transposable element. The one repeated sequence is found from −497 to −377, and is homologous to the *tourist* class of transposable ele-

ments in maize (BUREAU and WESSLER 1992). There is no reason to believe that this element is involved in paramutation. The presence of this element in *B-I* and *B′* is not surprising, as *tourist* elements have been found in one of every six sequenced maize genes, and are present in $>10^4$ copies per genome.

Another model that can be proposed for paramutation is one in which the two alleles directly interact by physical contact of proteins bound near or within the genes. This is a favored model for several types of allelic interaction in Drosophila: transvection (PIRROTTA 1990; GEYER, GREEN and CORCES 1990), *zeste-white* interaction (PIRROTTA 1990) and dominant position-effect variegation of *brown* (DREESEN, HENIKOFF and LOUGHNEY 1991). In these phenomena, a regulatory sequence in one allele can affect the expression of the gene on the other homologue. In the case of transvection, the regulation is positive; in the other cases the regulation is negative. This capacity is dependent on pairing; if the two alleles are not at homologous positions on the chromosomes, transregulation does not occur. COE (1966) examined the effect on paramutation of a translocation with a breakpoint proximal to *b* on chromosome 2. No effect was seen, but as the translocation is large and the breakpoint is several map units from *b*, it is not known whether this breakpoint would interfere with pairing at *b*.

We suggest a model in which a single allele can exist in two transcriptional states (*B′* and *B-I*) due to different complexes of proteins bound. The *B-I* state of high transcription is metastable; most gametes of a *B-I/B-I* plant are *B-I*, but a few are *B′*. Thus, the complex that is responsible for the *B-I* state, or a structure that promotes reassembly of the complex, may be capable of being inherited along with the *b* DNA through mitosis and meiosis. *B′* is generated spontaneously when the proteins rearrange into a more stable complex that results in less activation of transcription. The *B′* state is much more stable than the *B-I* state; spontaneous changes from *B′* to *B-I* are not seen (COE 1966; G. I. PATTERSON and V. L. CHANDLER, unpublished data). It may be that *B′* is the default state of the gene, and *B-I* represents a rare state that has been maintained by human selection. To explain the promotion of the change from *B-I* to *B′* by the presence of *B′* on the homologous chromosome, we propose that the two alleles interact via protein-protein contacts, and that this interaction promotes the formation of the more stable *B′* complex. The fact that a *B′/B-I* heterozygote is light and has a low level of *b* transcription, and the fact that a spontaneous change from *B-I* to *B′* can occur somatically, suggests that *b* paramutation is a somatic event that is subsequently inherited through mitosis and meiosis.

Work in Drosophila and yeast demonstrates the existence of mitotically heritable transcription states. In Drosophila, the hunchback protein negatively regulates expression of *ultrabithorax* by binding to a regulatory sequence (QIAN, CAPOVILLA and PIRROTTA 1991). However, this binding is transitory, and the suppression of *ultrabithorax* is observed many cell generations after the hunchback protein is gone (TAUTZ 1988; BENDER, TURNER and KAUFMAN 1987). Other examples of mitotically heritable expression states have been observed in Drosophila (PARO 1990). In yeast also, transcriptional states can be inherited (reviewed in LAURENSON and RINE 1992). In certain isogenic mutant strains, genes of the silent mating type loci can exist in an active or inactive state, and each state is heritable through mitosis. Switches from one state to the other can occur infrequently (PILLUS and RINE 1989; MAHONEY *et al.* 1991). Similarly, when genes are introduced into ectopic positions near a telomere, the genes are found in two heritable transcription states, and switch from one state to the other infrequently (GOTTSCHLING *et al.* 1990). These states are inherited in Drosophila and in yeast in the absence of DNA methylation; the involvement of heritable changes in chromatin have been proposed (PARO 1990; LAURENSON and RINE 1992). To explain paramutation, we propose that trans-interactions between alleles promote changes in expression states that are heritable through meiosis.

Trans-interactions have been seen in animals, most notably in Drosophila (TARTOF and HENIKOFF 1991; PIRROTTA 1990), but effects of these interactions are not transmitted to progeny. In plants, the germline and the soma are not separate lineages until very late in development; therefore, somatic events can be inherited. If transvection in animals and paramutation in plants are events that occur late in development, paramutation is more likely to be inherited, not because the mechanism is different, but because the organisms in which they occur develop differently. In animals, the cells that will give rise to gametes form a separate cell lineage very early in development. In Drosophila, this occurs at a stage when only approximately 1000 nuclei are present in the embryo (FULLILOVE and JACOBSON 1978), and most somatic cell fates are not yet established (INGHAM 1988). In maize, the meristem, a population of stem cells, produces most of the organs of the plant before the lineage that will produce gametes is separated from somatic lineages. Even very late sectors of somatic reversion, for example, sectors that consist of less than 1% of the somatic tissue of the male inflorescence, can be transmitted through the germline (DAWE and FREELING 1990).

Our model proposes that *B′* and *B-I* differ in chromatin structure. It may be possible to detect differences in chromatin structure by testing the sensitivity of sequences within the gene to nucleases; however, demonstrating that any changes are a cause, rather than an

effect of the change in transcription, will be difficult. The model predicts that regions that are important for regulation of *B'* and *B-I* transcription will also participate in paramutation. Experiments are in progress to genetically map the region within *B'* that is responsible for its ability to paramutate *B-I*, and for its tissue-specific RNA accumulation. Furthermore, if efforts to isolate mutants of *B'* or *B-I* defective in paramutation are successful, the model predicts that these mutants will be defective in some aspect of *b* transcription.

We thank DEVON BROWN for her assistance in cloning the *B-I* genomic sequences and for sequencing of *B-I* and *B'*. We are grateful to E. H. COE, JR. for sharing his genetic stocks and for discussing experiments with us. We thank K. CONE for sharing protocols for nuclei isolation and transcription analysis. We appreciate critical comments on the manuscript from KEN KUBO, ALICE BARKAN, CHARLES LAIRD, BRYAN PICKETT, TRUDEE TARKOWSKI, JEFF IRELAN, MANUEL SAINZ, TERRY SHROYER, MELISSA MITCHELL and MARC LESNICK. This work was supported by a National Institutes of Health grant (GM35791) to V.L.C.. G.I.P. was supported by an NIH predoctoral training grant (GM07413).

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Communicating editor: B. BURR