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Genetics

Molecular analysis of the maize anthocyanin regulatory locus *C1*

(transposon tagging/suppressor–mutator/enhancer–inhibitor)

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ABSTRACT **The *C1* gene of maize plays a regulatory role in the production of anthocyanin pigments in the aleurone layer of the endosperm. As an initial step toward understanding the molecular details of how *C1* controls pigment biosynthesis, we cloned the *C1* gene. This was accomplished by first cloning a mutable allele of *C1*, *c1-m5*, which contains the transposable element *Spm*. A combination of molecular and genetic analysis was used to identify the *Spm* at the *C1* locus. Individual genomic DNAs from a population in which the c1-mutable phenotype was segregating with the recessive c1 phenotype were digested with methyl-sensitive restriction enzymes and probed with a small DNA fragment derived from a defective *Spm*. One *Sal* I restriction fragment complementary to the *Spm* probe was shown to be present in the DNA of individuals with the c1-m5 phenotype but absent from DNA of individuals with a recessive c1 phenotype. Subsequent cloning and restriction analysis of this fragment revealed sequences flanking the *Spm* that proved to be *C1*-specific. A DNA fragment derived from the flanking sequences was then used as a probe to clone the wild-type *C1* gene and several additional alleles of *C1*, including one stable recessive, two mutations caused by *Ds* insertions, one mutation induced by insertion of a defective *Spm*, and two dominant mutations, *C1-S* and *C1-I*. RNA blot hybridization analysis of three *C1* alleles indicates that *C1* regulation of the *Bz1* and *A1* structural genes in the anthocyanin biosynthetic pathway is at the transcriptional level.**

The production of anthocyanin pigments in the aleurone layer of maize endosperm requires the products of both structural and regulatory genes (1, 2). *C1* is one of the regulatory genes, whose product appears to influence the coordinate regulation of the expression of at least two structural genes in the anthocyanin pathway: the *C2* gene, which encodes chalcone synthase, the first enzyme unique to the anthocyanin pathway (3), and *Bz1*, the structural gene for UDPglucose:flavonoid 3-*O*-glucosyltransferase, which catalyzes a glucosylation step late in the pathway (4). Presumably, expression of the other structural genes, *A1*, *A2*, and *Bz2*, is regulated in a like manner.

Kernels containing the dominant allele of *C1*, together with the dominant alleles of other genes in the anthocyanin pathway, are deeply pigmented. On the other hand, kernels that have the same genetic constitution but that are homozygous for the recessive allele, *c1*, are colorless. Other alleles of *C1*, some of which are due to transposable element insertions, have been described genetically. For example, one mutable allele of *C1*, *c1-m5* (5), arose by insertion of the transposable element *Spm* (suppressor–mutator; ref. 6), also known as *En* (enhancer; refs. 7 and 8), at the *C1* locus. Kernels containing *c1-m5* have a colorless aleurone with sectors of full pigmentation that are due to restoration of gene function by somatic excision of the element.

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Although *Spm* has recently been exploited as a transposon tag to clone several genes in maize (9–11), the gene isolations have not been simple. A principal difficulty is that there are many copies of sequences homologous to *Spm* in the maize genome. Some of these represent defective *Spm* (*dSpm*) elements that cannot transpose unless a transposition-competent *Spm* is present somewhere in the genome. For cloning purposes, molecular identification of the active *Spm* at the gene of interest, among the background of defective elements, has hitherto proved laborious.

To clone the *C1* gene, a strategy was used that allowed identification of the active *Spm* of *c1-m5*. An *Spm*-specific DNA fragment with relatively low copy number was used to probe genomic DNAs digested with methyl-sensitive restriction enzymes. A single *Sal* I fragment that both hybridized to the *Spm* probe and co-segregated with the c1-m5 phenotype in a backcross population was identified. Cloning and subsequent analysis of this fragment revealed that a short sequence adjacent to the *Spm* was specific for *C1*. This sequence was used as a hybridization probe to clone a wild-type *C1* gene. In addition, a number of mutant alleles were cloned, including several caused by transposable element insertions. Finally, the expression of *C1* and its role in regulating anthocyanin biosynthesis was examined by RNA blot analysis. Probes specific for two structural genes of the anthocyanin pathway, *Bz1* and *A1*, as well as *C1* itself, were hybridized to RNAs from kernels containing one of three *C1* alleles: *C1*, wild type; *C1-I*, a dominant allele that inhibits color expression; and *C1-S*, an allele that partially overcomes the inhibitory effect of *C1-I*.

MATERIALS AND METHODS

Plant Materials. The maize stocks used in these studies were obtained from B. McClintock. Unless otherwise noted, all loci carried the dominant alleles for anthocyanin biosynthesis. *C1*: normal dominant or wild-type allele, present in one of two combinations on the short arm of chromosome 9, *C1 Sh1 Bz1 Wx Ds* or *C1 sh1 bz1 wx*; *c1*: a stable recessive allele; *c1-m5*: a mutable allele arising from insertion of *Spm* at *C1* (5); *c1-m1* and *c1-m2*: mutable alleles arising from insertion of *Ds* at *C1* (12); *C1-I*: an inhibitor allele that is dominant to *C1*—i.e., in the triploid endosperm with the genetic constitution *C1-I/C1/C1*, the aleurone is colorless; *C1-S*: an allele that is partially dominant to *C1-I* when present in two copies in the endosperm—i.e., *C1-S/C1-S/C1-I* results in colored aleurone, whereas *C1-I/C1-I/C1-S* aleurone is colorless; *c1-Df4*: a stable recessive lethal allele [Seedlings are albescent and die at the three-leaf stage. This is a *Ds*-induced deletion of *C1* originally isolated by McClintock as a kernel with "reduced *C1-I*" expression from a cross of *C1-I Ds Sh1 Bz1, Ac* × *C1 sh1 bz1* (13, 14) and now present in the combination *c1-Df4 Ds sh1 Bz1 Wx Ds*]; *c1-m858*: a mutable allele isolated as a nonshrunken *c1*-mutable kernel from a cross of *c1 sh1 bz1 wx* × *C1 Sh1 bz1-m2 Wx, Spm* (this

Abbreviations: bp, base pair(s); kb, kilobase(s).

paper); *C1* revertant of *c1-m5*: a stable allele isolated as a fully colored kernel from a cross of *c1 sh1 bz1 wx* × *c1-m5 Sh1 Bz1 wx-m8* (this paper).

Nucleic Acid Isolation and Blot Hybridizations. Maize genomic DNA was prepared from young leaf tissue, blotted to nitrocellulose, and hybridized as described (15) except that all Southern hybridizations were performed at 50°C. Poly-$(A)^+$ RNA was prepared by a modification of the method of Kloppstech and Schweiger (16) from de-embryonated kernels harvested 30–32 days after pollination. Tissue (5 g) was ground to a fine powder in liquid nitrogen with a mortar and pestle. The powder was added to 25 ml of lysis buffer containing 0.1 M NaCl, 50 mM Tris·HCl (pH 7.4), 50 mM EDTA, 2% $NaDodSO_4$, proteinase K (200 μg/ml) (E.M. Biochemicals, Elmsford, NY), and stirred at room temperature for 10 min. Cell debris was pelleted by centrifugation in a Sorvall SS34 rotor at 10,000 rpm for 5 min, and the supernatant was extracted twice with an equal volume of chloroform:phenol:isoamyl alcohol (99/100/1) and once with chloroform:isoamyl alcohol (99/1). Sodium chloride (5 M) was added to the pooled aqueous phase to bring the NaCl concentration to 0.5 M, and poly$(A)^+$ RNA was prepared by passage over an oligo(dT)-cellulose column as described by Maniatis *et al.* (17). Electrophoresis of RNA on formaldehyde gels and transfer to nitrocellulose were according to Maniatis *et al.* (17) except that gels were stained with acridine orange (0.125 μg/ml) and photographed prior to transfer. Hybridizations were performed as described above except that incubation was for 18 hr at 42°C. For proper comparisons, the amounts of RNA loaded in each lane were normalized by prior hybridization with probes for the maize actin (18) and Waxy (*Wx*) (19) genes. Hybridization to actin-specific sequences should indicate how much mRNA (a combination of aleurone and endosperm transcripts) is present in each lane, and hybridization to Waxy should indicate the relative contribution of endosperm mRNA to the total.

Genomic Cloning. Approximately 10 μg of genomic DNA was digested overnight with 10–50 units of restriction enzyme using conditions specified by the suppliers (Bethesda Research Laboratories, New England Biolabs, Pharmacia). Digests were fractionated on 0.6% low melting point agarose gels and fragments of the desired size were excised from the gel and purified by passage over Elutips as described by the manufacturer (Schleicher & Schuell). *Sal* I fragments were cloned into the *Sal* I site of λgt*WES*·λB (20), and *Bgl* II fragments were cloned into the *Bam*HI site of λEMBL4 (21). Methods for packaging and screening recombinant phage were as described by Maniatis *et al.* (17). DNA sequencing was according to Maxam and Gilbert (22).

Plasmid Subclones and Probe Preparation. A 450-base-pair (bp) *Ban* II/*Xba* I fragment from the *dSpm* at *wx-m8* (23), a 250-bp *Sal* I/*Sau*3A fragment flanking the *Spm* from *c1-m5*, a 1-kilobase (kb) *Eco*RI fragment from *C1*, and a 2.1-kb *Pst* I fragment from the *Bz1* gene (24) were each cloned by appropriate staggered- or blunt-end ligation into the polylinker of pUC13 (25). A 1.5-kb *Bam*HI/*Hin*dIII fragment from the *Ac* element at *wx-m7* (26) was used as a *Ds*-specific probe. A 0.6-kb *Pst* I fragment from the *A1* gene (9) was used to probe RNA blots. Fragments to be used as probes were prepared by double digestion of plasmid subclones and purification from low melting point agarose gels as described above. Probes were labeled with ^{32}P by nick-translation (15) or random primed labeling (27).

RESULTS

Cloning Strategy. Cloning of *C1* initially focused on the *c1-m5* allele, which arose as a result of insertion of the transposable element *Spm* at *C1*. To minimize the amount of screening necessary to distinguish the active *Spm* element associated with the *C1* locus from other *Spm*-like sequences in the genome, we adopted two approaches. First, as a probe we chose a small fragment of the *dSpm* from *wx-m8* (see *Materials and Methods*) that, in contrast to most of the sequences in *Spm*, is represented in the genome only ≈20 times (unpublished data). Second, we took advantage of the observation made by others (28–30) that the DNA sequences in and around active transposable elements appear to be undermethylated and therefore are more subject to cleavage by methyl-sensitive restriction enzymes than normally methylated maize sequences. Thus, after digestion by these enzymes, DNAs associated with inactive *Spm*-like sequences persist as high molecular weight species that can be ignored in subsequent analyses.

To identify the *Spm* responsible for the c1-m5 phenotype, we examined individuals from a population in which *c1-m5* was segregating with *c1*. From the backcross *c1-m5 Sh1 Bz1 wx-m8/c1 sh1 bz1 wx* × *c1 sh1 bz1 wx*, two kernel phenotypes were obtained: c1-mutable, colorless aleurone with pigmented sectors; and c1, colorless aleurone. Genomic DNAs, prepared from individual plants grown from c1-mutable and colorless kernels, were digested with the methyl-sensitive restriction enzyme *Sal* I and probed with the *Spm* probe derived from *wx-m8*. The results of the analysis are shown in Fig. 1. As expected, most of the sequences homologous to the probe are contained in DNA that is poorly digested with *Sal* I and therefore does not migrate far into the gel. One restriction fragment, ≈8.3 kb, is present as a strongly hybridizing band in all the lanes containing DNA from plants grown from c1-mutable kernels but is absent in lanes containing DNA from plants grown from colorless kernels. In some lanes, a 2.1-kb band is present; this band represents the *Sal* I fragment that contains a portion of the *dSpm* at *wx-m8*, which is segregating in this material.

The 8.3-kb *Sal* I fragment was cloned into the vector λgt*WES*·λB and analyzed by restriction mapping. The map of the *Sal* I fragment, shown as a bold line on the map of *c1-m5* in Fig. 2, is similar to those of other cloned *Spm* elements (9–11, 31). Notice that the 8.3-kb fragment contains an internal *Sal* I site that is not cleaved in genomic DNA (see *Discussion*). Comparison of the DNA sequence of the left end of the 8.3-kb fragment (data not shown) with the published

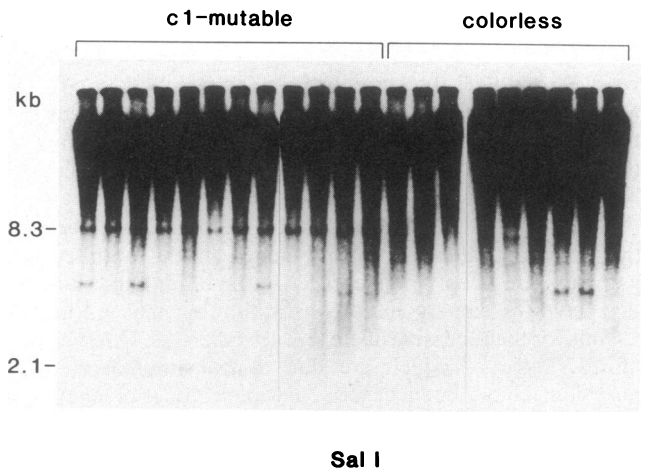

FIG. 1. Molecular identification of the *Spm* associated with the c1-m5 phenotype. Genomic DNAs were prepared from individual plants grown from c1-mutable or colorless progeny kernels of the backcross *c1-m5 Sh1 Bz1 wx-m8/c1 sh1 bz1 wx* × *c1 sh1 bz1 wx*. After digestion with *Sal* I, the DNA was fractionated on agarose gels, transferred to nitrocellulose, and hybridized with a probe derived from the *dSpm* at *wx-m8*. Sizes of relevant restriction fragments are indicated.

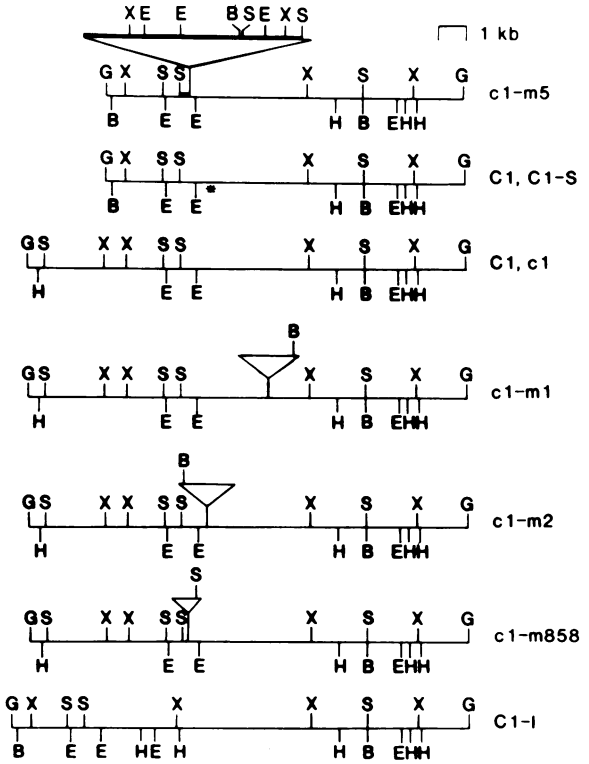

FIG. 2. Restriction endonuclease cleavage site maps of *Bgl* II fragments from different *C1* alleles. The 8.3-kb *Sal* I fragment cloned from *c1-m5* is indicated by a bold line. All the maps, with the exception of that for *c1-m5*, were generated by comparative digests of cloned and genomic DNAs. The map for *c1-m5* was deduced from the structure of previously cloned *Spm* elements and from hybridization of genomic DNA with probes specific for *C1*; the site of insertion of the *Spm* element was determined as described in the text. The asterisk marks the position of a *Pst* I site mentioned in the text. Restriction sites: B, *Bam*HI; E, *Eco*RI; G, *Bgl* II; H, *Hin*dIII; S, *Sal* I; X, *Xba* I.

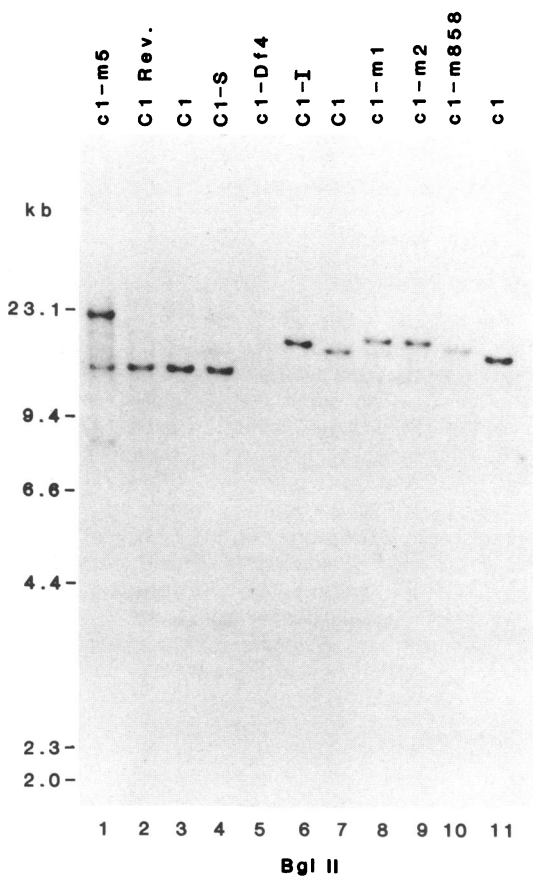

FIG. 3. Southern hybridization analysis of different *C1* alleles. Genomic DNAs were fractionated on agarose gels, transferred to nitrocellulose, and hybridized with a probe derived from sequences flanking the *Spm* element cloned from *c1-m5*. DNA in each lane contained the indicated allele of *C1*. *C1* Rev. refers to a full-colored revertant of *c1-m5*. The wild-type *C1* alleles in lanes 3 and 7 were from two stocks containing *C1 Sh1 Bz1 wx* and *C1 Sh1 Bz1 Wx Ds*, respectively. Sizes of the marker fragments included in the gel are indicated.

sequence at the end of the *dSpm* at *wx-m8* (23) revealed that the cloned fragment extends ≈350 bp beyond the *Spm*.

Identification of Cloned Sequences as *C1*. A 250-bp *Sal* I/*Sau*3A fragment excised from the sequence adjacent to *Spm* was subcloned from the 8.3-kb *Sal* I fragment. If this fragment represented a portion of the *C1* locus, then if it were used to probe *Bgl* II digests of genomic DNAs from plants carrying various *C1* alleles, the following could be predicted: First, since there are no *Bgl* II sites in the probe, only one band representative of *C1* should hybridize strongly in any one lane, and, in general, that is the case as shown in Fig. 3. The notable exception is shown in the lane corresponding to *c1-m5*, which contains two major bands of ≈21 and ≈12.5 kb. The lower band is the same size as the band present in a fully colored revertant of *c1-m5* and presumably represents DNA from which the *Spm* has excised somatically. Second, since many of the alleles analyzed here represent insertions of transposable elements into the *C1* locus, the sizes of hybridizing bands should vary for these alleles. That is the result obtained. Interestingly, this analysis shows a restriction fragment length polymorphism between two wild-type *C1* alleles (lanes 3 and 7), both in McClintock stocks. Finally, if the probe represents *C1*, there should be no hybridization to DNA from a line that has a deletion of the gene. There is no hybridization signal in lane 5, which contains DNA from *c1-Df4*, a mutant in which *C1* has been deleted.

Analysis of Molecular Structure of *C1*. By using the 250-bp *Sal* I/*Sau*3A fragment as a *C1*-specific probe, a 12.5-kb *Bgl* II fragment was cloned from genomic DNA containing a wild-type *C1* gene. Based on comparative restriction digests of cloned and genomic DNAs, this cloned fragment corresponds to the *Bgl* II fragments shown in Fig. 3 (lanes 2 and 3). The restriction map of the cloned DNA, as shown in Fig. 2 (row 2), is in agreement with the *C1* map reported earlier by Paz-Ares *et al.* (10). The site of insertion of the *Spm* in *C1* was resolved by probing blots containing appropriate restriction enzyme digests of the cloned wild-type allele with the 250-bp *Sal* I/*Sau*3A fragment from *c1-m5* (row 1).

To determine what parts of the wild-type clone represented single-copy sequences likely to be part of *C1* itself, as opposed to repeated sequences commonly found flanking genes, several subfragments (1–2 kb long) were tested as probes on blots of genomic DNA (data not shown). Only a small continuous portion of the cloned 12.5-kb *Bgl* II fragment appears to be unique in the genome; this region includes the 1-kb *Eco*RI fragment and extends to a *Pst* I site (indicated by an asterisk in Fig. 2) located ≈0.5 kb to the right of the *Eco*RI fragment. The analysis does not rule out the existence of very small regions of single-copy DNA elsewhere in the cloned *Bgl* II fragment. The 1-kb *Eco*RI fragment was subcloned and used as a hybridization probe in subsequent experiments.

To begin to define the sequences important for the expression and regulation of the *C1* locus, a number of *C1* alleles were cloned, including several insertional mutations that alter gene expression. Another wild-type allele, from a line containing *C1 Sh1 Bz1 Wx* with *Ds* at its standard position proximal to *Wx*, was cloned as a 15.5-kb *Bgl* II fragment and

showed the restriction site polymorphism expected from the Southern analysis (Fig. 3, lanes 3 and 7). The polymorphism was confined to the region of repeated DNA to the left of the unique *Eco*RI fragment (compare rows 2 and 3 in Fig. 2). Two *c1* mutants, *c1-m1* and *c1-m2*, which were derived from this wild-type progenitor and characterized by McClintock as transpositions of *Ds* from its standard position to the *C1* locus, were also cloned. Restriction analysis of these two clones and hybridization with a *Ds*-specific probe verified that the mutant DNAs contain *Ds* insertions of 2.2 and 2.5 kb for *c1-m1* and *c1-m2*, respectively (Fig. 2, rows 4 and 5). Note that the orientation of the two *Ds* insertions is opposite (refer to positions of the *Bam*HI sites within the elements). The structure of another insertional mutation, *c1-m858*, is shown in Fig. 2 (row 6). This mutation was isolated from a cross in which one of the parents carried an active *Spm* element. Although the genetic analysis of this mutant has not been completed, restriction mapping and hybridization with an *Spm*-specific probe show that this mutation is due to the insertion of a 1.1-kb *dSpm* within the 1-kb *Eco*RI fragment. The position of the *Sal* I site in this element, compared to the position of the *Sal* I site near the end of the full-length *Spm* element in *c1-m5* (compare rows 1 and 6), indicates that these two insertions are in the same relative orientation. This orientation is the same as that reported for two other cloned *Spm* (*En*) insertions at *C1* (10).

Two other cloned *C1* alleles proved to be indistinguishable from wild type on the basis of restriction mapping. A recessive allele, *c1*, yields a digestion pattern identical to the 15.5-kb *C1* clone (Fig. 2, row 3), and the dominant *C1-S* allele yields a pattern similar to the 12.5-kb *C1* clone (row 2).

Analysis of the restriction map of the *C1-I* clone (row 7) shows that restriction sites at the ends of the cloned fragment are identical to those of the 12.5-kb *C1* clone (row 2). *C1-I* also contains a 1-kb *Eco*RI fragment that is homologous to the same size fragment from wild type. However, to the right of this fragment, there are additional sequences comprising ≈3.5 kb. These sequences appear to represent an insertion, whose precise nature and location have not been determined. Preliminary analysis indicates that these extra sequences are present in only one or two copies in the genome (data not shown). Whether insertion of these sequences has also been accompanied by some rearrangement of *C1*-related sequences remains to be shown.

Transcription of *C1*. Expression of *C1* was examined by analyzing the RNAs produced by three alleles of *C1*: the wild-type allele; the dominant inhibitor of color, *C1-I*; and *C1-S*, an allele that causes more anthocyanins to be produced and can overcome the inhibition by *C1-I* (see *Materials and Methods*). Poly(A)$^+$ RNA, prepared from developing kernels 30–32 days after pollination, was analyzed by RNA blot hybridization (Fig. 4). Approximately equal amounts of RNA were loaded in each lane, as described in *Materials and Methods*. The blot shown in Fig. 4 (*Left*) was probed with the 1-kb *Eco*RI fragment specific for *C1*. The center lane shows that there are two main transcripts, ≈1.5 and ≈1.2 kb, produced by *C1*. These RNAs are similar in size to the 1.6- and 1.4-kb RNAs described by Paz-Ares *et al.* (10); however, a 0.3-kb *C1*-specific RNA, which they reported, is not detected here. Transcripts of the same sizes as in wild type are also present in *C1-S* (left lane); however, these transcripts appear to be more abundant. A larger RNA (≈2.5 kb) is also noted. The lane representing *C1-I* shows the presence of two transcripts, slightly different in size from those of wild type. The intensity of the hybridization signal in *C1-I* is higher than that in wild-type *C1*.

To confirm the genetic evidence that suggests that *C1* plays a regulatory role in anthocyanin biosynthesis, blots of RNAs from *C1*, *C1-S*, and *C1-I* were hybridized with probes specific for two structural genes of the anthocyanin pathway, *Bz1* and *A1*. The results for *Bz1* (Fig. 4 *Center*) show that *C1-S* kernels produce more *Bz1* RNA than wild-type *C1* kernels. Furthermore, no *Bz1* RNA is detectable in kernels from a line containing *C1-I*. Similar results were obtained when the *A1*-specific probe was used (Fig. 4 *Right*).

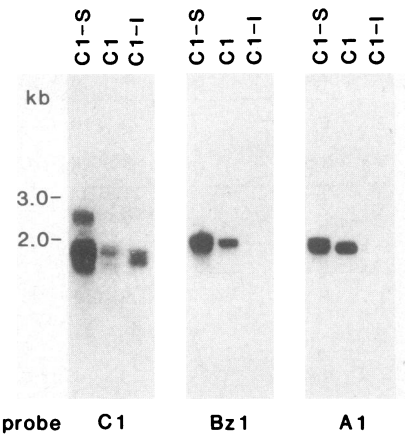

FIG. 4. RNA blot hybridization analysis of transcripts from different *C1* alleles. Poly(A)$^+$ RNAs were prepared from developing kernels harvested 30–32 days after pollination from plants in which all loci for aleurone anthocyanin synthesis, except *C1*, carried the dominant alleles; *C1* genotypes were as indicated. RNAs (≈5 μg per lane) were fractionated on formaldehyde agarose gels and transferred to nitrocellulose. Blots were probed with a 1-kb *Eco*RI fragment specific for *C1* (*Left*), a 2.1-kb *Pst* I fragment specific for *Bz1* (*Center*), and a 0.6-kb *Pst* I fragment specific for *A1* (*Right*). Sizes of marker RNAs included in the gel are indicated.

DISCUSSION

The strategy used to clone *C1* combines molecular procedures and traditional genetic analyses and should prove especially useful for cloning maize genes tagged with high-copy-number transposable elements. The method relies on the observation that the DNA sequences in and around active transposable elements seem to be undermethylated (28–30). Consequently, such sequences are more susceptible to cleavage by methyl-sensitive restriction enzymes than are the bulk of highly methylated maize DNA sequences. We have found, however, that the undermethylation may not be uniform throughout the sequence of the transposable element itself. Nonetheless, use of one or more methyl-sensitive enzymes in Southern analyses of populations segregating for mutable phenotypes should allow identification of the active elements responsible for those phenotypes.

Previous assignment of a regulatory role for *C1* in pigment formation in the aleurone had been based on the effects of various *C1* mutations on the expression of structural genes in the anthocyanin biosynthetic pathway. Our analysis of the mRNA levels of two of these structural genes, *Bz1* and *A1*, in kernels that carry *C1*, *C1-I*, or *C1-S* supports earlier conclusions. In particular, the lack of *Bz1* hybridization to mRNA from kernels homozygous for *C1-I* agrees with earlier measurements of the protein product of the *Bz1* gene that showed that the level in aleurone homozygous for *C1-I* is only 3% of the wild-type level (4). In contrast, the level of *Bz1* mRNA was higher than wild type in kernels homozygous for *C1-S*, as might have been expected from the C1-S phenotype in which aleurone pigmentation is darker than normal. The levels of *A1* mRNA appear to be affected in the same manner—i.e., decreased by *C1-I* and increased by *C1-S*—when compared to wild-type *C1*. Taken together, these results underscore the regulatory role of *C1* and suggest that *C1* regulation of these two structural genes of the anthocyanin pathway is at the level of transcription. Furthermore,

these data clearly indicate that the products of *C1-S* and *C1-I* are functionally distinct in their regulation of *Bz1* and *A1*; compared to the effect of wild-type *C1*, *C1-S* increases expression of *Bz1* and *A1* alleles, whereas *C1-I* inhibits their expression altogether.

The analysis of the transcription of *C1* itself may now provide additional clues about the functional relationship among *C1* alleles, especially among wild type, *C1-S*, and *C1-I*. Phenotypically, C1-I has been somewhat of a puzzle. Unlike the negative dominant mutations isolated for some prokaryotic genes—e.g., the *lac* repressor of *Escherichia coli* (32) and the *tet* repressor of Tn*10* (33)—that show partial dominance in the presence of the wild-type genes, the phenotype of *C1-I* is almost completely dominant to the wild type. Aleurones of the genetic constitution *C1-I/C1/C1* have either no color or only a faint blush. This dosage-independent behavior of *C1-I* may be explained, in part, by the significantly higher *C1*-specific mRNA levels in *C1-I* kernels as compared to wild type. If the product of *C1-I* competes in some way with the product of the wild-type gene, as has been suggested earlier (34), then the apparently higher level of *C1-I* expression could result in overproduction of the competing molecule and thus inhibit function of the wild-type product. This hypothesis predicts that *C1-I* would exhibit partial dominance in the presence of the "super" allele *C1-S* that produces an amount of RNA comparable to that produced by *C1-I*. That prediction is fulfilled by comparison of the dosage dependence in endosperms heterozygous for *C1-I* and *C1-S*: the aleurone of *C1-S/C1-S/C1-I* is pale colored, whereas that of *C1-I/C1-I/C1-S* is colorless.

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1. Coe, E. H., Jr., & Neuffer, M. G. (1977) in *Corn and Corn Improvement*, ed. Sprague, G. F. (Am. Soc. Agron., Madison, WI), pp. 111–224.
2. Dooner, H. K. (1982) in *Maize for Biological Research*, ed. Sheridan, W. F. (University Press, Grand Forks, ND), pp. 123–128.
3. Dooner, H. K. (1983) *Mol. Gen. Genet.* **189,** 136–141.
4. Dooner, H. K. & Nelson, O. E., Jr. (1977) *Biochem. Genet.* **15,** 509–519.
5. McClintock, B. (1963) *Carnegie Inst. Washington Yearb.* **62,** 486–493.
6. McClintock, B. (1954) *Carnegie Inst. Washington Yearb.* **53,** 254–260.
7. Peterson, P. A. (1953) *Genetics* **38,** 682–683.
8. Peterson, P. A. (1965) *Am. Nat.* **99,** 391–398.
9. O'Reilly, C., Shepherd, N. S., Pereira, A., Schwarz-Sommer, Z., Bertram, I., Robertson, D. S., Peterson, P. A. & Saedler, H. (1985) *EMBO J.* **4,** 877–882.
10. Paz-Ares, J., Wienand, U., Peterson, P. A. & Saedler, H. (1986) *EMBO J.* **5,** 829–833.
11. Wienand, U., Weydemann, U., Niesbach-Klosgen, U., Peterson, P. A. & Saedler, H. (1986) *Mol. Gen. Genet.* **203,** 202–207.
12. McClintock, B. (1948) *Carnegie Inst. Washington Yearb.* **47,** 155–169.
13. McClintock, B. (1953) *Carnegie Inst. Washington Yearb.* **52,** 227–237.
14. McClintock, B. (1954) *Carnegie Inst. Washington Yearb.* **53,** 254–260.
15. Evola, S. V., Burr, F. A. & Burr, B. (1986) *Theor. Appl. Genet.* **71,** 765–771.
16. Kloppstech, K. & Schweiger, H. G. (1976) *Cytobiologie* **13,** 394–400.
17. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
18. Shah, D. M., Hightower, R. C. & Meagher, R. B. (1983) *J. Mol. Appl. Genet.* **2,** 111–126.
19. Shure, M., Wessler, S. & Fedoroff, N. (1983) *Cell* **35,** 225–233.
20. Leder, P., Tiemeier, D. & Enquist, L. (1977) *Science* **196,** 175–177.
21. Frischauf, A.-M., Lehrach, H., Poustka, A. & Murray, N. (1983) *J. Mol. Biol.* **170,** 827–842.
22. Maxam, A. M. & Gilbert, W. (1980) *Methods Enzymol.* **65,** 499–560.
23. Gierl, A., Schwarz-Sommer, Z. & Saedler, H. (1985) *EMBO J.* **4,** 579–583.
24. Fedoroff, N., Furtek, D. & Nelson, O. E., Jr. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 3825–3829.
25. Yanisch-Perron, C., Vieira, J. & Messing, J. (1985) *Gene* **33,** 103–119.
26. Behrens, U., Fedoroff, N., Laird, A., Muller-Neumann, M., Starlinger, P. & Yoder, J. (1984) *Mol. Gen. Genet.* **194,** 346–347.
27. Feinberg, A. P. & Vogelstein, B. (1984) *Anal. Biochem.* **137,** 266–267.
28. Fedoroff, N., Wessler, S. & Shure, M. (1983) *Cell* **35,** 235–242.
29. Dellaporta, S. L. & Chomet, P. S. (1985) in *Plant Gene Research: Genetic Flux in Plants*, eds. Hohn, B. & Dennis, E. S. (Springer, New York), pp. 170–217.
30. Chandler, V. L. & Walbot, V. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 1767–1771.
31. Pereira, A., Schwarz-Sommer, Z., Gierl, A., Bertram, I., Peterson, P. A. & Saedler, H. (1985) *EMBO J.* **4,** 17–23.
32. Miller, J. H. (1978) in *The Operon*, eds. Miller, J. H. & Reznikoff, W. S. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 31–88.
33. Isackson, P. J. & Bertrand, K. P. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 6226–6230.
34. McClintock, B. (1951) *Carnegie Inst. Washington Yearb.* **50,** 174–181.