Intragenic Transposition of *Ac* Generates a New Allele of the Maize *P* Gene

Thomas Peterson

Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724

Manuscript received May 7, 1990
Accepted for publication July 6, 1990

ABSTRACT

The maize *P* gene is required for the production of red phlobaphene pigments in the pericarp and cob. The *P-vv* allele, which specifies variegated pericarp and cob, contains an insertion of the transposable element *Ac* in the *P* gene. A new *P-ovov* allele (*o*range *v*ariegated pericarp and cob) was obtained as a single event mutation from *P-vv*. In contrast to the progenitor *P-vv* allele, *P-ovov* provides substantial pericarp and cob pigmentation and produces significant amounts of normal-sized *P* transcripts. The *Ac* element is not detectably altered in the *P-ovov* allele, but it has transposed to a new position within *P* that is 161 bp distant and inserted in the opposite orientation. This example provides molecular confirmation for the hypothesis that changes in expression of genes bearing insertions of transposable elements can occur via movement of the element to new sites within the gene.

McCLINTOCK recognized that transposable elements can profoundly alter, in several ways, the expression of genes into which they are inserted (McCLINTOCK 1951, 1956, 1965a; for reviews see FINCHAM and SASTRY 1974; FEDOROFF 1983, 1989; NEVERS, SHEPHERD and SAEDLER 1986). A transposable element can affect the level of gene expression, and it also can produce specific mutational events which result in clonal sectors of cells exhibiting altered gene function. Both the level of expression and the occurrence of mutational events can be modulated during development. For each transposable element-induced allele, the combination of these expressions results in a distinctive phenotypic pattern (McCLINTOCK 1951, 1956, 1965a, 1967). Such patterns are most evident in those cases in which transposable elements are associated with genes, such as the loci in maize which are involved in kernel pigmentation, where subtle changes in gene expression can be discerned. McCLINTOCK also determined that the characteristic pattern of expression can be altered even though the transposable element remains at the affected locus. Derivatives of unstable alleles can exhibit changes in the timing and/or frequency of mutational events, and the level of gene expression (McCLINTOCK 1947, 1951, 1955, 1965a); such derivatives can result from deletions within the transposable element sequences (SCHIEFELBEIN *et al.* 1985, 1988a; SCHWARZ-SOMMER *et al.* 1985, 1987; TACKE *et al.* 1986; MASSON *et al.* 1987; FEDOROFF *et al.* 1988). The expression of genes containing transposable element insertions is also affected by alterations in the activity of the element at the locus, or, in cases in which a non-autonomous element is present at the locus, the activity of its autonomous counterpart (McCLINTOCK 1958, 1964, 1965b). Alterations in element activity have been correlated with methylation or modification of element sequences (BENNETZEN 1985, 1987; CHANDLER and WALBOT 1986; SCHWARTZ and DENNIS 1986; CHOMET, WESSLER and DELLAPORTA 1987). Changes in the pattern of expression are also suspected to occur as the result of movement of a transposable element to new positions within a gene [BRINK and WILLIAMS 1973; PETERSON 1976, 1977; SCHIEFELBEIN *et al.* 1988b; M. A. JOHNS, personal communication (*Maize Genetics Cooperation Newsletter* **64:** 20)]. In one case, a change in expression of the *wx-m7* allele, which contains an insertion of the transposable element *Ac* in the maize *Waxy* gene, was caused by movement of *Ac* from the promoter region to the ninth exon of *Waxy* (P. CHOMET, personal communication). Here, we report that a very small shift in position of *Ac* resulted in a striking alteration in expression of the maize *P* gene.

MATERIALS AND METHODS

Maize stocks: The *P* gene is involved in the production of a red pigment (phlobaphene) in the pericarp and the soft floral parts of the cob (STYLES and CESKA 1977) (see Figure 1). The pericarp is the outer covering of the maize kernel; it is the remnant of the ovary wall and hence is maternal tissue. The pigmented floral parts include the glumes, lemma and palea, which subtend the ovary and make up the chaff of the mature cob (MILLER 1919). *P* alleles are commonly identified by a 2-letter suffix describing their expression in the pericarp and cob; as shown in Figure 1, *P-rr* specifies red pericarp and cob, *P-ww* specifies white (colorless) pericarp and cob, and *P-wr* specifies white (colorless) pericarp and red cob. In heterozygotes, color in any portion of the pericarp and cob is dominant to colorless (ANDERSON 1924).

The inbred line W23 (genotype *P-wr*) was obtained from

the Maize Genetics Cooperation Stock Center, Urbana, Illinois. The inbred line 4Co63 (genotype *P-ww*) was obtained from the National Seed Storage Laboratory, Fort Collins, Colorado. Stocks carrying *P-vv*, and the ear with the original orange variegated pericarp sector, were obtained from TONY PRYOR, Canberra, Australia. The *P-ovov* allele described here (*P-ovov-1114*) is of independent origin from the *P-ovov* types previously studied by VALENTINE and BRINK [F. VALENTINE, personal communication (*Maize Genetics Cooperation Newsletter*, **31:** 129 and **34:** 118)]. The *Ac* tester stock *R-sc:124* variant 4 was obtained from J. KERMICLE, Madison, Wisconsin.

Test for *Ac* activity in *P-ovov* stocks: Two sibling plants (2069–3 and 2069–4) were grown from kernels within the original sector of kernels with orange variegated pericarp (see below). These sibling plants were of *P-ovov/P-wr r/r* genotype and were pollinated with pollen from *P-ww/P-ww r/r* plants. The resulting kernels were sown, and a total of 248 progeny plants were tested for *P* expression and the presence or absence of *Ac*. *P* expression was determined by scoring the pericarp and cob pigmentation of the ears produced by each plant. To test for *Ac*, pollen from each plant was placed on silks of plants of the maize stock *R-sc:124* variant 4. The test-crossed ears produced by the *R-sc:124* variant 4 plants were scored for the presence of colorless aleurone sectors, which indicate that an active *Ac* was present in the pollen parent (see below). Plants whose pollen produced approximately 50% or more kernels with colorless aleurone sectors on the testcrossed ears were scored as *Ac+*. Plants whose pollen produced few or no sectored kernels were scored as *Ac−*. Results of these tests are presented in Table 1.

R-sc:124, the progenitor of *R-sc:124* variant 4, conditions purple pigmentation to the aleurone layer of maize kernels, while *r* gives colorless aleurone. *R-sc:124* variant 4 contains a chromosome-breaking *Ds* element located in chromosome 10 between *R-sc* and the centromere. In the absence of *Ac*, chromosomes carrying the *Ds* insertion are stable and full aleurone pigmentation results. When the stock is crossed with a line carrying *Ac*, chromosome 10 frequently breaks at the site of *Ds* insertion. This breakage results in the loss of *R-sc* on an acentric fragment, and production of a colorless aleurone sector. Thus the use of the *R-sc:124* variant 4 stock is analogous to the wellknown *C Ds* stock of MCCLINTOCK, in which chromosome breakage at *Ds* in response to *Ac* and consequent loss of the color factor *C* results in colorless aleurone sectors (J. KERMICLE, personal communication).

Analysis of pericarp pigments: Mature dried seeds from plants homozygous for *P-ovov* or *P-rr* were soaked in water for several hours, and the pericarps were peeled off and ground to a powder in liquid nitrogen. Pericarp pigments were extracted with 1% hydrochloric acid/methanol and analyzed on a Beckman DU-40 spectrophotometer.

DNA isolation, mapping and Southern blot analysis: Genomic leaf DNA was isolated from young leaves of individual plants in the 8–10 leaf stage by a previously described method (SHURE, WESSLER and FEDOROFF 1983). Genomic DNA (2.5 μg) was digested with restriction enzymes, electrophoresed through 0.6% agarose gels, and transferred to nitrocellulose according to the method of SOUTHERN (1975). Filters were prehybridized for 4–6 hr and hybridizations were carried out overnight at 42° in 50% formamide, 5 × SSC, 1 × Denhardt's solution, 15 mM NaH_2PO_4, 10% dextran sulfate and 250 μg/ml heat-denatured salmon sperm DNA.

Genomic cloning: Southern analysis indicated that the *Ac* elements at the *P-vv* and *P-ovov* loci were contained on approximately 6-kb *Bgl*II restriction fragments. Genomic DNA from plants carrying *P-vv* or *P-ovov* was digested with *Bgl*II, electrophoresed on a 0.7% agarose gel, and DNA fragments in the 5–7-kb size range were isolated using NA45 DEAE membrane (Schleicher and Schuell). The *Bgl*II termini of the genomic fragments were partially filled in with dATP and dGTP using DNA polymerase I, Klenow fragment (IBI). The resulting partially filled in fragments were ligated to bacteriophage lambda ZAP (Stratagene) which had been digested with *Xho*I and partially filled in with dTTP and dCTP. Ligations, *in vitro* packaging and plaque hybridizations were done according to standard procedures (MANIATIS, FRITSCH and SAMBROOK 1982). The partial libraries were screened with a *P*-locus probe flanking *Ac* which was obtained from a previously cloned genomic fragment from *P-vv* (LECHELT *et al.* 1989). Phagemids containing the genomic inserts from *P-vv* or *P-ovov* were isolated by the Lambda ZAP Automatic Excision protocol (Stratagene). Nucleotide sequences were determined by the dideoxy chain termination method (SANGER, MICKLEN and COULSON 1977).

RNA isolation and Northern blot analysis: Total RNA was prepared from pericarps dissected from developing kernels at 21 days after pollination. Purification of polyadenylated RNA, gel electrophoresis and Northern hybridizations were performed as previously described (LECHELT *et al.* 1989).

RESULTS AND DISCUSSION

Origin and characterization of *P-ovov*: The *P-vv* allele, which specifies variegated pericarp and cob (Figure 1), comprises the transposable element *Ac* inserted into the *P* gene (BARCLAY and BRINK 1954; LECHELT *et al.* 1989). A single dose of *P-vv*, in the absence of additional copies of *Ac* in the genome, gives pericarp with a relatively large number of red sectors; each red sector derives from the progeny of a single cell in which *P-vv* reverted to *P-rr* by excision of *Ac* (BRINK and NILAN 1952). The pattern of expression of a single *P-vv* allele, in the absence of other genomic *Ac* elements, is known as medium variegated pericarp. The presence of an additional *Ac* element, either in a second *P-vv* allele or elsewhere in the genome, causes a delay in timing of excision of *Ac* from *P-vv*, and gives a pattern termed light variegated pericarp (BRINK and NILAN 1952).

From *P-vv*, we obtained an allele with the strikingly different pattern of expression termed *P-ovov* (orange variegated pericarp and cob; see below). *P-ovov* was detected as a spontaneous mutation from *P-vv* as follows. A plant of *P-vv/P-wr* genotype was grown and pollinated with pollen from a plant of *P-wr* genotype. The resulting ear had medium variegated pericarp and red cob, except for a sector of approximately 40 kernels with orange variegated pericarp and red cob. The sharp boundary between the orange variegated pericarp and the medium variegated pericarp indicates that the mutation of *P-vv* to *P-ovov* occurred as a single event.

Mutations of *P* which produce sectors of altered pericarp pigmentation can be recovered from the

FIGURE 1.—Pericarp and cob pigmentation specified by the following *P* alleles (from left to right): *P-rr*, *P-ww*, *P-wr*, *P-vv*, and *P-ovov*. The plants producing these ears were heterozygous with the *P-ww* allele. The rightmost ear, expressing the *P-ovov* allele, has a sector of 9 kernels at the base of the ear with altered pericarp pigmentation. In multikernel sectors the tip region of the kernel, near the silk attachment point, is often pigmented, as it is here. This unusual pigmentation pattern is under further investigation (P. ATHMA and T. PETERSON, unpublished results).

kernels within such a sector, because the pericarp and the egg cell are of related lineage (ANDERSON and BRINK 1952). Six progeny plants were grown from kernels within the original sector of orange variegated kernels; four progeny plants produced ears with colorless pericarp, red cob (genotype *P-wr/P-wr*), and two progeny plants produced ears with orange variegated pericarp, red cob (genotype *P-ovov/P-wr*). Ten plants were grown from the medium variegated kernels outside the orange variegated sector; six gave ears with variegated pericarp and red cob (*P-vv/P-wr*) and four produced ears with colorless pericarp, red cob (*P-wr/P-wr*). Although the number of progeny plants examined is not large, the results are in accord with Mendelian segregation of the *P-ovov* and *P-wr* alleles among the kernels in the original orange variegated sector, and the *P-vv* and *P-wr* alleles in the portion of the ear with medium variegated pericarp.

P-ovov conditions a very deep orange pericarp and cob pigmentation (right-hand ear in Figure 1) which, upon cursory examination, resembles the uniform red pigmentation specified by *P-rr* (left-hand ear in Figure 1). However, closer inspection of kernels expressing *P-ovov* reveals the presence of sectors of darker red pericarp which are distinguishable from the adjacent orange pericarp, especially near the base of the kernel (Figure 2). This orange variegated phenotype resembles that previously described for several *P-ovov* alleles derived independently from *P-vv* (F. VALENTINE, personal communication (*Maize Genetics Cooperation Newsletter*, **31:** 129 and **34:** 118). An additional component of the orange variegated phenotype is the occurrence of sectors of pale or colorless tissue; these occur commonly as small sectors, as shown in Figure 2, or less commonly as large multikernel sectors, as shown in Figure 1. Sectors of light or colorless pericarp are extremely rare on kernels expressing *P-rr* (P. ATHMA and T. PETERSON, unpublished results).

To determine whether the orange color of *P-ovov* pericarps is due to the production of a pigment different from that specified by *P-rr*, pigments were extracted from mature pericarps from plants homozygous for *P-ovov* and for *P-rr*, and the extracts were compared by UV/visible spectroscopy. The spectral profiles of the extracts from *P-ovov* and *P-rr* pericarps were qualitatively similar, and dilution of a *P-rr* pigment extract produced a spectral profile nearly identical to that given by the *P-ovov* extract (C. BROWN and T. PETERSON, unpublished results). These results indicate that the orange color of *P-ovov* pericarps derives not from synthesis of a new pigment, but rather from a reduced level of the pigments specified by *P-rr*.

FIGURE 2.—Pericarp pigmentation specified by the progenitor *P-vv* allele (upper) and the derivative allele *P-ovov* (lower). Each allele was heterozygous with a *P* allele specifying colorless pericarp. Note that *P-vv* specifies colorless pericarp with red sectors, while *P-ovov* specifies orange pericarp with frequent red sectors and occasional light sectors.

The genetic properties of the *P-ovov* allele were investigated in progeny of the cross *P-ovov/P-wr* × *P-ww/P-ww*. Because the pericarp and cob are maternal tissues, the kernels produced by this cross bore the orange variegated pericarp specified by the maternal genotype (*P-ovov/P-wr*). These kernels were sown, and the resulting plants were expected to consist of the *P-ovov/P-ww* and *P-wr/P-ww* genotypes in approximately equal numbers. For each progeny plant, the pericarp and cob color of the ear was scored, and the presence or absence of *Ac* was determined in testcrosses.

Three points can be drawn from the results (Table 1). First, the *P-ovov* allele is unstable, as evidenced by the ten progeny plants which produced ears with red pericarp and cob (P-rr) and three plants which produced ears with variant pericarp and cob pigmentation. Of the three variant plants, one produced ears with colorless pericarp and cob, and two produced ears with lighter orange pericarp and cob. The plants

TABLE 1

Segregation of *Ac* activity with the *P-ovov* allele in the progeny of the cross: *P-ovov/P-wr* × *P-ww/P-ww*

Cross	Presence of *Ac*	Ear pigmentation			
		wr	ovov	rr	Other
2069-3 × *P-ww*	+	0	27	0	0
	−	46	0	1	0
2069-4 × *P-ww*	+	2	82	5	2
	−	78	0	4	1
Total of both crosses:	+	2	109	5	2
	−	124	0	5	1
Total by ear pigmentation:		126		122	

See MATERIALS AND METHODS for details.

which produced the P-rr and variant ears are presumed to carry *P* alleles derived from mutation of *P-ovov*, since the *P-wr* allele used in this cross is stable; the germinal instability of *P-ovov* evidenced here is consistent with the continued presence of an *Ac* element in the *P-ovov* allele. Second, the number of plants which produced P-wr ears (126) is approximately equal to the combined number of plants which produced P-ovov, P-rr, and variant ears (122). This shows that *P-ovov* segregates as an allele of *P-wr*; the P-ovov phenotype does not depend upon unlinked modifying factors. Third, *Ac* activity is tightly linked to *P-ovov*. 109 plants producing P-ovov ears carried *Ac*; there were no examples of plants producing P-ovov ears which did not carry *Ac*. Although the number of plants tested was not large, the results of these genetic tests indicated that the *P-ovov* allele carried an *Ac* element inserted within or near the *P* gene; the molecular tests described below proved that this was in fact the case.

Has the *Ac* element at the *P* locus undergone a compositional change in the mutation of *P-vv* to *P-ovov*? This possibility was tested by comparing the abilities of the *Ac* elements associated with the *P-vv* and *P-ovov* alleles to activate a chromosome-breaking *Ds* element (*Dissociation*; non-autonomous element which can transpose and/or cause chromosome breaks in the presence of a *trans*-acting *Ac*). No differences in the frequency or developmental timing of the *Ds*-response were seen, suggesting that the *trans*-acting functions of *Ac* are unchanged (T. PETERSON, unpublished results).

***Ac* is transposed and inverted in *P-ovov*:** Southern analysis of DNA from plants carrying *P-vv* and *P-ovov* shows that the *Ac* element associated with *P-ovov* is located near the site where *Ac* is present in *P-vv*, but that the *Ac* element is in an inverted orientation in *P-ovov* compared to *P-vv* (Figures 3 and 4). As can be seen in Figure 3, DNA from *P-vv* plants digested with *Bam*HI and hybridized with a *P* locus probe gives bands at 11 and 10 kb, whereas DNA from *P-ovov* plants gives bands at 10 and 7 kb. The common 10

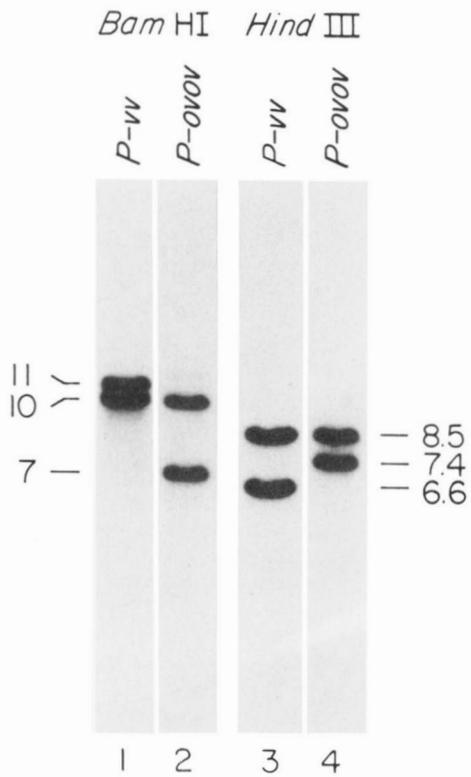

FIGURE 3.—Southern analysis of genomic DNA from plants homozygous for the progenitor *P-vv* allele and for the derivative allele *P-ovov*. Samples in lanes 1 and 2 were digested with *Bam*HI, and those in lanes 3 and 4 were digested with *Hin*dIII. The probe is a 400 bp *Sac*I-*Sal*I fragment from the *P* locus flanking the *Ac* element (Figure 4). This probe cross-hybridizes with a linked repeated sequence which gives the 10-kb *Bam*HI band and the 8.5-kb *Hin*dIII band (LECHELT *et al.* 1989).

kb band results from cross-hybridization of the *P* probe with a linked repeated sequence present in both *P-vv* and *P-ovov* (LECHELT *et al.* 1989). The 11-kb band in *P-vv* results from cutting at a *Bam*HI site in the *P* locus approximately 7 kb from the site of *Ac* insertion, and at the single *Bam*HI site in *Ac*; the *Bam*HI site in *Ac* is located 184 bp from one end of the 4.5-kb *Ac* element (POHLMAN, FEDOROFF and MESSING 1984). In *P-ovov* plants, the 11-kb band is replaced by a 7-kb band which results from inversion of the *Ac* element relative to its position in *P-vv* (Figure 4). In a *Hin*dIII digest, the *P* probe detects a band of 8.5 kb in both *P-vv* and *P-ovov* due to cross-hybridization with the linked repeated sequence (LECHELT *et al.* 1989). The *Hin*dIII digest also gives bands of 6.6 and 7.4 kb for *P-vv* and *P-ovov*, respectively; the difference in size is primarily a result of the opposite orientations of *Ac* in each allele (Figure 4).

Genomic fragments from the *P-vv* and *P-ovov* alleles were cloned and the nucleotide sequences at the sites of *Ac* insertion were determined. As shown in Figure 5, the *Ac* element in the *P-vv* allele has excised, transposed 161 bp, and reinserted in the opposite orientation to give the *P-ovov* allele.

Insertion of *Ac* or *Ds* transposable elements is known to result in a duplication of 8 bp of the target site DNA. It is surprising that the *Ac* element in *P-vv* is not flanked by a duplication; however, 8 bp direct repeats are present at the new location of *Ac* in *P-ovov* (Figure 5). It seems probable that an 8-bp duplication was formed when *Ac* initially inserted in the *P* gene to form the *P-vv* allele, but that this repeat was subsequently deleted. Since the *P-vv* allele is of longstanding origin (EMERSON 1917) and the progenitor *P* allele of *P-vv* is not identified, this hypothesis is difficult to test directly. Previous reports have documented the occurrence of deletions adjacent to *Ds* (MCCLINTOCK 1953, 1954) and *Ac* (DOONER, RALSTON and ENGLISH 1988). Since the *Ac* element in *P-vv* is most likely located within an intron (LECHELT *et al.* 1989), a small adjacent deletion including the 8-bp direct repeat would not be expected to eliminate the capacity for *P* gene expression upon excision of *Ac*.

The frequent occurrence of *Ac* excisions from *P-vv*, in which *Ac* is not bordered by an 8-bp duplication, indicates that excision of *Ac* does not require the presence of flanking duplicated sequences as specific sites for endonucleolytic cleavage prior to excision, as was previously proposed (PEACOCK *et al.* 1984; SAEDLER and NEVERS 1985). DOONER, ENGLISH and RALSTON (1988) have also shown that efficient transposition of *Ac* does not require a flanking duplication. Additionally, there is a transversion of two base pairs at the site of *Ac* excision in the *P-ovov* allele (Figure 5); similar transversions are frequently found at the sites of excision of *Ds* and other transposable elements (PEACOCK *et al.* 1984; COEN *et al.* 1989). Such transversions are thought to be generated during element excision either by template switching of a repair DNA polymerase (SAEDLER and NEVERS 1985), or by "offset" nicking of a transient hairpin formed by ligation of the host DNA ends after element excision (COEN *et al.* 1989). Our results show that transposition can still occur by an apparently normal transversion-generating mechanism despite the absence of a flanking duplication.

RNA expression from *P-ovov*: Using the *Ac* element as a transposon tag we have previously isolated 34 kb of genomic DNA from the *P* locus (LECHELT *et al.* 1989). We have identified a 6.6-kb region which produces multiple transcripts in the fully functional *P-rr* allele; the *P-vv* allele produces very low levels of the *P-rr*-specific RNAs, and instead a chimeric transcript containing both *P* and *Ac* sequences, and terminating within *Ac*, is found (LECHELT *et al.* 1989). In contrast, RNA from the *P-ovov* allele contains a significant level of transcripts of the same size as two of the five transcripts found in *P-rr* (Figure 6). These *P-ovov* transcripts are detected using as probes fragments of the *P* locus located on both sides of the *Ac* insertion, indicating that the new site of *Ac* insertion

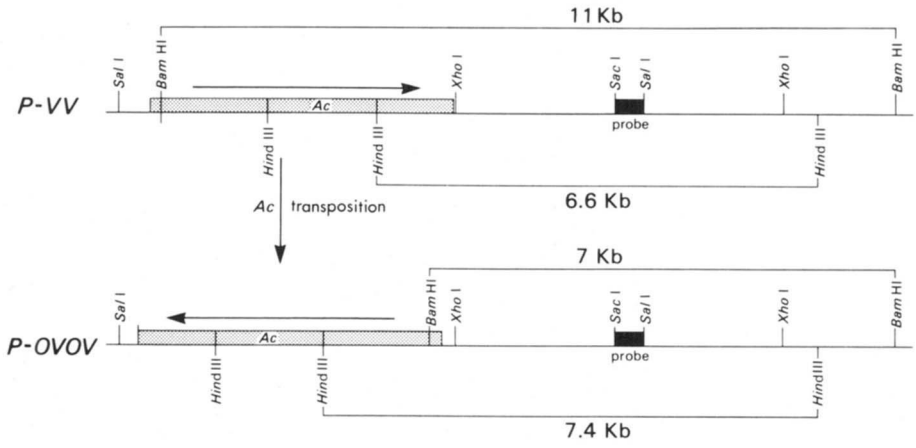

FIGURE 4.—Map of the *P-vv* and *P-ovov* alleles showing fragments obtained by digestion with *Bam*HI and *Hin*dIII. The 11- and 7-kb *Bam*HI bands and the 7.4- and 6.6-kb *Hin*dIII bands arise due to the different orientations of the *Ac* element in the *P-vv* and *P-ovov* alleles.

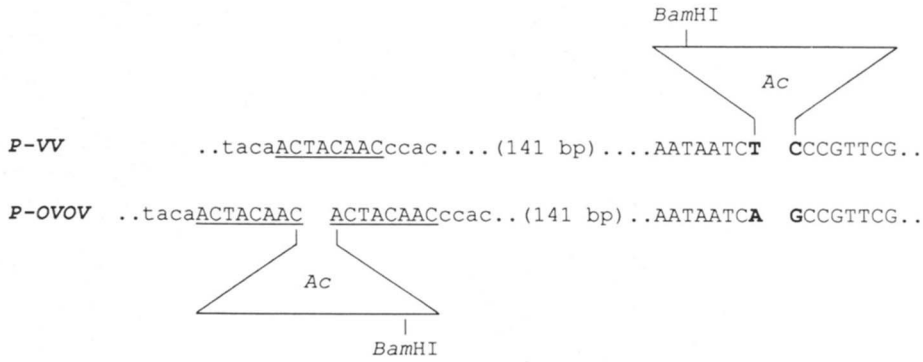

FIGURE 5.—DNA sequences at the sites of *Ac* insertions in *P-vv* (upper) and *P-ovov* (lower). The *Ac* element is indicated by a triangle; the *Bam*HI site, which is near the 5′ end of the *Ac* transcript (KUNZE *et al.* 1987) indicates the opposite orientations of *Ac* in *P-vv* and *P-ovov*. Complementary transversions at the site of *Ac* excision in the *P-ovov* allele are in bold (**A** and **G**). The underlined sequence *ACTACAAC* is present once in *P-vv* and duplicated in *P-ovov*.

and/or the inverted orientation of *Ac* in the *P-ovov* allele allows most or all of the *Ac* element to be spliced out of the RNA.

These observations relate to proposed mechanisms for splicing of RNA transcripts of several maize genes into which transposable elements have inserted (KIM *et al.* 1987; WESSLER, BARAN and VARAGONA 1987; DENNIS *et al.* 1988; RABOY *et al.* 1989). Previous studies (WESSLER, BARAN and VARAGONA 1987; DENNIS *et al.* 1988) have shown that transcription of alleles of the maize *waxy* and *Adh1* genes containing *Ds* element insertions can proceed through the *Ds* elements resulting in chimeric transcripts. Most of the *Ds* element sequences are then removed from the RNA by splicing reactions which join splice donor sequences present within *Ds*, near one end of the element, to 3′ splice acceptor sites located within the gene, downstream of the *Ds* insertion. These splicing reactions can result in the removal of most of the *Ds* sequences from the RNA and the production of partially functional protein(s). A prediction of this model is that splicing of *Ac* or *Ds* element sequences should occur only when the element is in one orientation within the affected gene (such that the 3′ end of *Ac* or *Ds* is nearer the 5′ end of the gene carrying the element insertion). With *P-vv* and *P-ovov*, pigmentation and the production of normal sized *P* transcripts is correlated with the orientation of the *Ac* element in the manner predicted by the splicing model. Alternatively, the differences in *P* expression between *P-vv* and *P-ovov* could result from different sites of insertion of *Ac* with respect to the intron/exon structure of the *P* gene. However, the location of the *Ac* elements in *P-vv* and *P-ovov* in an intron of the *P* gene is supported by Northern analysis of RNA from *P-rr* pericarps using as hybridization probes DNA fragments immediately flanking the *Ac* element in *P-vv* (LECHELT *et al.* 1989).

Based on the above results, the following model is proposed to explain the different components of the orange variegated phenotype specified by *P-ovov*. The orange pericarp is composed of cells in which *Ac* remains inserted within the *P* transcribed region; in these cells *Ac* sequences would be spliced from the *P* transcript, resulting in a moderate level of *P* expression. The numerous red sectors are explained by clones of cells in which *Ac* has excised from *P-ovov*, thereby restoring a *P-rr* allele; excision of *Ac* has been confirmed in a *P-rr* revertant allele derived from *P-ovov* (T. PETERSON, unpublished results). Finally, the occasional sectors of light pericarp derive from cells in which mutations of *P-ovov* have occurred, including deletions (P. ATHMA and T. PETERSON, unpublished results) and further intragenic transpositions (T. PETERSON, unpublished results). Genetic experiments have previously shown that *Ac* tends to transpose to nearby sites (MCCLINTOCK 1956; GREENBLATT 1984; DOONER and BELACHEW 1989; SCHWARTZ 1989). Our results demonstrate that *Ac* can transpose very short molecular distances within a gene to generate new

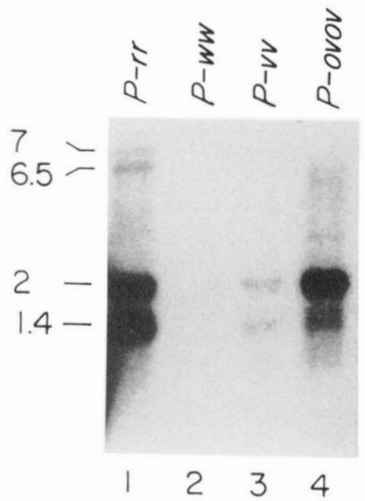

FIGURE 6.—Northern analysis of pericarp RNA from plants homozygous for different *P* alleles. The RNA source in each lane is: lane 1, *P-rr-4B2* (a *P-rr* revertant from *P-vv*); lane 2, *P-ww-1112* (a *P-ww* allele derived from *P-vv*; *P-ww-1112* carries a 12.5-kb deletion including most of the region transcribed in *P-rr* (P. ATHMA and T. PETERSON, unpublished results); lane 3, *P-vv*; lane 4, *P-ovov*. The probe used is a 600 bp *Sac*I fragment located 2 kb to the right of the site of *Ac* insertion in the *P-ovov* allele as drawn in Figure 4 (Probe #14 in LECHELT *et al.* 1989). The same pattern of hybridizing transcripts is seen using as probe a fragment from the *P* locus located 3 kb to the left of the *Ac* insertion site in the *P-ovov* allele as drawn in Figure 4 (a fragment of probe #8 in LECHELT *et al.* 1989).

alleles. The phenotype specified by each allele is likely determined by both the site of insertion and the orientation of the element; some intragenic transpositions could go unnoticed if the new site or orientation of the element does not specify a different phenotype.

I thank B. MCCLINTOCK and W. J. PEACOCK for critical readings of the manuscript, TONY PRYOR for the ear carrying the original sector of kernels with orange variegated pericarp, J. KERMICLE for the *Ac* tester stock *R-sc:124* variant 4, SUSAN ALLAN for technical assistance, CHRISTOPHER BROWN for performing the pigment analyses, GREG GIBSON for field assistance, and W. J. PEACOCK and LIZ DENNIS of Commonwealth Scientific and Industrial Research Organization, Division of Plant Industry, for discussions and support. This work was supported by a National Science Foundation grant to T.A.P., a National Institutes of Health grant (RO1 GM39832) to T.A.P., and a grant from Pioneer Hi-Bred International, Inc.

LITERATURE CITED

ANDERSON, E. G., 1924 Pericarp studies in maize. II. The allelomorphism of a series of factors for pericarp color. Genetics **9**: 442–453.

ANDERSON, R. E., and R. A. BRINK, 1952 Kernel pattern in variegated pericarp maize and the frequency of self-colored offspring. Am. J. Bot. **39**: 637–644.

BARCLAY, P. C., and R. A. BRINK, 1954 The relation between *Modulator* and *Activator* in maize. Proc. Natl. Acad. Sci. **40**: 1118–1126.

BENNETZEN, J. L., 1985 The regulation of *Mutator* function and *Mu1* transposition. UCLA Symp. Mol. Cell. Biol. **35:** 343–353.

BENNETZEN, J. L., 1987 Covalent DNA modification and the regulation of *Mutator* element transposition in maize. Mol. Gen. Genet. **208:** 45–51.

BRINK, R. A., and R. A. NILAN, 1952 The relation between light variegated and medium variegated pericarp in maize. Genetics **37**: 519–544.

BRINK, R. A., and E. WILLIAMS, 1973 Mutable *R-navajo* alleles of cyclic origin in maize. Genetics **73:** 273–296.

CHANDLER, V. L., and V. WALBOT, 1986 DNA modification of a maize transposable element correlates with loss of activity. Proc. Natl. Acad. Sci. USA **83:** 1767–1771.

CHOMET, P. S., 1988 Change in state of the *wx-m7* allele caused by intragenic transposition of the maize controlling element, *Activator*. Ph.D. thesis, State University of New York, Stony Brook, N.Y.

CHOMET, P. S., S. WESSLER and S. L. DELLAPORTA, 1987 Inactivation of the maize transposable element *Activator* is associated with its DNA modification. EMBO J. **6:** 295–302.

COEN, E. S., T. P. ROBBINS, J. ALMEIDA, A. HUDSON and R. CARPENTER, 1989 Consequences and mechanisms of transposition in *Antirrhinum majus*, pp. 413–436 in *Mobile DNA*, edited by D. E. BERG and M. M. HOWE. AMERICAN SOCIETY FOR MICROBIOLOGY, WASHINGTON, D.C.

DENNIS, E. S., M. M. SACHS, W. L. GERLACH, L. BEACH and W. J. PEACOCK, 1988 The *Ds1* transposable element acts as an intron in the mutant allele *Adh1-Fm335* and is spliced from the message. Nucleic Acids Res. **16:** 3815–3828.

DOONER, H. K., and A. BELACHEW, 1989 Transposition pattern of the maize element *Ac* from the *bz-m2(Ac)* allele. Genetics **122:** 447–457.

DOONER, H. K., J. ENGLISH and E. J. RALSTON, 1988 The frequency of transposition of the maize element *Activator* is not affected by an adjacent deletion. Mol. Gen. Genet. **211:** 485–491.

DOONER, H. K., E. RALSTON and J. ENGLISH, 1988 Deletions and breaks involving the borders of the *Ac* element in the *bz-m2(Ac)* allele of maize, pp. 213–226 in *Plant Transposable Elements*, edited by O. NELSON. Plenum, New York.

EMERSON, R. A., 1917 Genetical studies of variegated pericarp in maize. Genetics **2**: 1–35.

FEDOROFF, N. V., 1983 Controlling elements in maize, pp. 1–63 in *Mobile Genetic Elements*, edited by J. A. SHAPIRO. Academic Press, Orlando, Fla.

FEDOROFF, N. V., 1989 Maize transposable elements, pp. 375–411 in *Mobile DNA*, edited by D. E. BERG and M. M. HOWE. American Society for Microbiology, Washington, D.C.

FEDOROFF, N., P. MASSON, J. BANKS and J. KINGSBURY, 1988 Positive and negative regulation of the *Suppressor-Mutator* element, pp. 1–15 in *Plant Transposable Elements*, edited by O. NELSON. Plenum, New York.

FINCHAM, J. R. S., and G. R. K. SASTRY, 1974 Controlling elements in maize. Annu. Rev. Genet. **8:** 15–50.

GREENBLATT, I. M., 1984 A chromosome replication pattern deduced from pericarp phenotypes resulting from movements of the transposable element, *Modulator*, in maize. Genetics **108:** 471–485.

KIM, H.-Y., J. W. SCHIEFELBEIN, V. RABOY, D. B. FURTEK and O. E. NELSON, JR., 1987 RNA splicing permits expression of a maize gene with a defective *Suppressor-mutator* transposable element insertion in an exon. Proc. Natl. Acad. Sci. USA **84:** 5863–5867.

KUNZE, R., U. STOCHAJ, J. LAUFS and P. STARLINGER, 1987 Transcription of transposable element *Activator* (*Ac*) of *Zea mays* (L.) EMBO J. **6**: 1555–1563.

LECHELT, C., T. PETERSON, A. LAIRD, J. CHEN, S. DELLAPORTA, E. DENNIS, P. STARLINGER and W. J. PEACOCK, 1989 Isolation and molecular analysis of the maize *P* locus. Mol. Gen. Genet. **219:** 225–234.

MANIATIS, T., E. F. FRITSCH and J. SAMBROOK, 1982 *Molecular*

Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

MASSON, P., R. SUROSKY, J. A. KINGSBURY and N. V. FEDOROFF, 1987 Genetic and molecular analysis of the *Spm-dependent a-m2* alleles of the maize *a* locus. Genetics **177:** 117–137.

MCCLINTOCK, B., 1947 Cytogenetic studies of maize and neurospora. Carnegie Inst. Wash. Year Book **46**: 146–152.

MCCLINTOCK, B., 1951 Chromosome organization and genic expression. Cold Spring Harbor Symp. Quant. Biol. **16:** 13–47.

MCCLINTOCK, B., 1953 Mutation in maize. Carnegie Inst. Wash. Year Book **52**: 227–237.

MCCLINTOCK, B., 1954 Mutation in maize and chromosomal aberrations in Neurospora. Carnegie Inst. Wash. Year Book **53**: 254–260.

MCCLINTOCK, B., 1955 Controlled mutation in maize. Carnegie Inst. Wash. Year Book **54**: 245–255.

MCCLINTOCK, B., 1956 Controlling elements and the gene. *Cold Spring Harbor Symp. Quant. Biol.* **22:** 197–216.

MCCLINTOCK, B., 1958 The *Suppressor-mutator* system of control of gene action in maize. Carnegie Inst. Wash. Year Book **57:** 415–429.

MCCLINTOCK, B., 1964 Aspects of gene regulation in maize. Carnegie Inst. Wash. Year Book **63:** 592–602.

MCCLINTOCK, B., 1965a The control of gene action in maize. Brookhaven Symp. Biol. **18**: 162–184.

MCCLINTOCK, B., 1965b Components of action of the regulators *Spm* and *Ac*. Carnegie Inst. Wash. Year Book **64:** 527–536.

MCCLINTOCK, B., 1967 Regulation of pattern of gene expression by controlling elements in maize. Carnegie Inst. Wash. Year Book **65:** 568–578.

MILLER, E. C., 1919 Development of the pistillate spikelet and fertilization in *Zea mays* L. J. Agric. Res. **18:** 255–265.

NEVERS, P., N. S. SHEPHERD and H. SAEDLER, 1986 Plant transposable elements. Adv. Bot. Res. **12:** 103–203.

PEACOCK, W. J., E. S. DENNIS, W. L. GERLACH, M. M. SACHS and D. SCHWARTZ, 1984 Insertion and excision of *Ds* controlling elements in maize. Cold Spring Harbor Symp. Quant. Biol. **49:** 347–354.

PETERSON, P. A., 1976 Basis for the diversity of states of controlling elements in maize. Mol. Gen. Genet. **149:** 5–21.

PETERSON, P. A., 1977 The position hypothesis for controlling elements in maize, pp. 429–435 in *DNA Insertion Elements, Plasmids and Episomes*, edited by A. J. BUKHARI, J. A. SHAPIRO and S. L. ADHYA. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

POHLMAN, R. F., N. V. FEDOROFF and J. MESSING, 1984 The nucleotide sequence of the maize controlling element *Activator*. Cell **37:** 635–643.

RABOY, V., H.-Y. KIM, J. W. SCHIEFELBEIN and O. E. NELSON, JR., 1989 Deletions in a *dSpm* insert in a maize *bronze-1* allele alter RNA processing and gene expression. Genetics **122:** 695–703.

SAEDLER, H., and P. NEVERS, 1985 Transposition in plants: a molecular model. EMBO J. **4**: 585–590.

SANGER, F., S. MICKLEN and A. R. COULSON, 1977 DNA sequencing with chain terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**: 5463–5467.

SCHIEFELBEIN, J. W., V. RABOY, N. V. FEDOROFF and O. E. NELSON, 1985 Deletions within a defective *Suppressor-mutator* element in maize affect the frequency and developmental timing of its excision from the *bronze* locus. Proc. Natl. Acad. Sci. USA **82:** 4783–4787.

SCHIEFELBEIN, J. W., V. RABOY, H.-Y. KIM and O. E. NELSON, 1988a Molecular characterization of *Suppressor-mutator* (*Spm*)-induced mutations at the *bronze-1* locus in maize: the *bz-m13* alleles, pp. 261–278 in *Plant Transposable Elements*, edited by O. NELSON. Plenum, New York.

SCHIEFELBEIN, J. W., D. B. FURTEK, H. K. DOONER and O. E. NELSON, JR., 1988b Two mutations in a maize *bronze-1* allele caused by tranposable elements of the *Ac-Ds* family alter the quantity and quality of the gene product. Genetics **120**: 767–777.

SCHWARTZ, D., 1989 Pattern of *Ac* transposition in maize. Genetics **121:** 125–128.

SCHWARTZ, D., and E. DENNIS, 1986 Transposase activity of the *Ac* controlling element in maize is regulated by its degree of methylation. Mol. Gen. Genet. **205:** 476–482.

SCHWARZ-SOMMER, Z., A. GIERL, R. BERNDTGEN and H. SAEDLER, 1985 Sequence comparison of 'states' of *a1-m1* suggests a model of *Spm* (*En*) action. EMBO J. **4:** 2439–2443.

SCHWARZ-SOMMER, Z., N. SHEPHERD, E. TACKE, A. GIERL, W. ROHDE, L. LECLERCQ, M. MATTES, R. BERNDTGEN, P. A. PETERSON and H. SAEDLER, 1987 Influence of transposable elements on the structure and function of the *A1* gene of *Zea mays*. EMBO J. **6:** 287–294.

SHURE, M., S. WESSLER and N. FEDOROFF, 1983 Molecular identification and isolation of the *waxy* locus in maize. Cell **35**: 225–233.

SOUTHERN, E., 1975 Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**: 503–517.

STYLES, E. D., and O. CESKA, 1977 The genetic control of flavonoid biosynthesis in maize. Can. J. Genet. Cyt. **19**: 289–302.

TACKE, E., Z. SCHWARZ-SOMMER, P. A. PETERSON and H. SAEDLER, 1986 Molecular analysis of states of the *A1* locus of *Zea mays*. Maydica **31:** 83–91.

WESSLER, S. R., G. BARAN and M. VARAGONA, 1987 The maize transposable element *Ds* is spliced from RNA. Science **237:** 916–918.

Communicating editor: B. BURR