Transposition of *Ac* From the *P* Locus of Maize Into Unreplicated Chromosomal Sites

Jychian Chen,* Irwin M. Greenblatt† and Stephen L. Dellaporta*

**Department of Biology, Yale University, New Haven, Connecticut 06511, and †Department of Molecular and Cell Biology, University of Connecticut, Storrs, Connecticut 06268*

Manuscript received April 10, 1987
Accepted May 30, 1987

ABSTRACT

We have analyzed donor and target sites of the mobile element *Activator* (*Ac*) that are altered as a result of somatic transposition from the *P* locus in maize. Previous genetic analysis has indicated that the two mitotic daughter lineages which result from *Ac* transposition from *P* differ in their *Ac* constitution at the *P* locus. Both lineages, however, usually contain transposed *Ac* elements which map to the same genetic position. Using methylation-sensitive restriction enzymes and genomic blot analysis, we identified *Ac* elements at both the donor *P* locus and *Ac* target sites and used this assay to clone the *P* locus and to identify transposed *Ac* elements. Daughter lineages were shown to be mitotic descendants from a single transposition event. When both lineages contained *Ac* genetic activity, they both contained a transposed *Ac* element on identical genomic fragments independent of the genetic position of the target site. This indicates that in the majority of cases, *Ac* transposition takes place after replication of the donor locus but before completion of replication at the target site.

THE *P* locus of maize, located on the short arm of chromosome *1*, conditions pigmentation of the pericarp and cob tissues. This locus provides a unique system for studying somatic mutational events for several reasons. The visible pigmentation patterns determined by the *P* locus are developmentally innocuous. Also, somatic cells which give rise to the pericarp covering a single kernel share a common lineage with the underlying germ cell except for an intervening meiotic division (ANDERSON and BRINK 1952). Hence, *P* mutations that are visible in the pericarp tissue can easily be selected for and recovered from the underlying germ line cells for subsequent genetic and molecular analysis. Moreover, since pericarp is somatic tissue, the fate of each strand of DNA from a progenitor cell can be followed by recovering contiguous somatic daughter lineages in kernel progeny underlying the somatic sectors.

Variegated pericarp, the phenotype of the unstable *P-vv* allele (EMERSON 1914), is caused by the insertion of the mobile element *Activator* (*Ac*) (previously referred to as *Mp*) into the *P* locus (BRINK and NILAN 1952; BARCLAY and BRINK 1954). The variegation is exhibited as red stripes on an otherwise colorless pericarp background. The colorless tissue results from *Ac* suppression of *P* gene action and each red stripe results from somatic excision of *Ac* from *P*. This excision event usually restores pigmentation potential in the mitotic descendants of a cell where *Ac* excises from *P*. The pattern of striping is dependent on the dosage of *Ac* (BRINK and NILAN 1952). Heavy striping (called medium variegated pericarp) is exhibited when a single *Ac* at *P* is heterozygous with a recessive colorless pericarp allele (*P-ww* or *P-wr*) (BRINK and NILAN 1952). The addition of a second *Ac*, independent of its location, reduces the rate of striping to a discrete phenotype called light variegated pericarp.

Contiguous sectors of light variegated and red pericarp often arise on otherwise medium variegated ears and are referred to as twin mutations. Twin mutations are thought to result from a single transposition of *Ac* from the *P* locus during ear development. The mitosis following the somatic transposition of *Ac* results in one daughter cell lacking an *Ac* at *P* (potentially a red sector) and the other daughter cell containing two doses of *Ac*: the recently transposed *Ac* in addition to the *Ac* at *P* (potentially a light variegated sector) (BRINK and NILAN 1952). When paired clones derived from these daughter cells are included in the pericarp, twin mutations are formed.

Two classes of twin mutations can be classified on the basis of whether or not *Ac* activity is present in the red sector (GREENBLATT and BRINK 1962). Type I twins contain *Ac* activity in the red sector; while type II twins lack such activity. In type I twins, *Ac* activity in the red sector maps to the same genetic position as the transposed *Ac* element in the cotwin light variegated sector (GREENBLATT and BRINK 1962). To account for these results, it was postulated that transposition of *Ac* from *P* involves the excision of *Ac* from one chromatid after replication of the donor locus and integration into a target site that has not yet

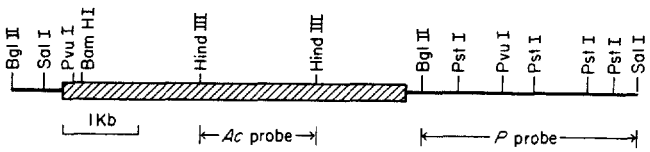

FIGURE 1.—Schematic representation of the 8.0-kb *Sal*I fragment of *P-vv*. A restriction map of the 8.0-kb *Sal*I band was prepared from the cloned fragment. The position of *Ac* is shown as the *cross-hatched rectangle*. The *Ac* probe which represents the 1.6-kb internal *Hin*dIII fragment of *Ac* was gel purified from the cloned *Ac9* element (FEDOROFF, WESSLER and SHURE 1983). The *P* probe is the 2.5-kb *Bgl*II-*Sal*I fragment gel purified from the 8.0-kb *Sal*I clone.

completed replication at the time of insertion (GREENBLATT and BRINK 1962; GREENBLATT 1966, 1968, 1974, 1984). Completion of chromosomal replication and mitotic segregation will then result in a transposed *Ac* element located at identical chromosomal positions in both red and light variegated cotwin lineages.

In this paper, we report the molecular analysis of donor and target sites for *Ac* in type I twin mutations. Our results provide direct evidence for the mechanism of *Ac* transposition outlined by GREENBLATT (1984). Using a molecular approach, we can examine cases where *Ac* transposes to linked as well as unlinked receptor sites; the latter events have not been resolved by genetic analysis. Our results show that *Ac* elements, independent of their chromosomal location, can be identified by genomic blot analysis using methylation-sensitive restriction enzymes. Using this assay, we cloned a region of the *P* locus containing *Ac*. We also show that type I twin mutations contain transposed *Ac* elements found on the same genomic fragment in red and light variegated cotwin progeny regardless of the position of the transposed *Ac* in relation to the *P* locus. These results provide molecular confirmation that in type I twins *Ac* transposition from *P* involves excision of *Ac* from one chromatid of a replicated donor site to a target site that has not yet replicated.

MATERIALS AND METHODS

Genetic stocks: The *P-vv* allele was originally identified by EMERSON (1914) and backcrossed at least eight times into the inbred lines W22, W23 and 4Co63. The 4Co63 line is homozygous for the *P-ww* allele that conditions colorless pericarp and cob tissues. W22 and W23 are homozygous for the *P-wr* allele which gives a colorless pericarp and colored cob phenotype. W23 and 4Co63 inbred lines are homozygous for a recessive seed color allele of the *R* locus (*r-g*). Progeny from light-variegated and red twin sectors were the same materials used in the study of GREENBLATT (1984). The cotwin numbers in the figures refer to the original designation of these mutations.

The genetic test for *Ac* activity in red and light variegated progeny consisted of pollinating red or light-variegated progeny ears with pollen from a W22 tester line homozygous for *P-wr* and a *Ds*-suppressed *R-sc* allele designated *r-sc:m3* (kindly supplied by J. KERMICLE, University of Wisconsin). *R-sc* confers strong uniform pigmentation to the aleurone layer of kernels. The *r-sc:m3* allele gives a colorless aleurone in the absence of *Ac* activity and a variegated colored aleurone in the presence of an active *Ac* element.

DNA preparation, restriction and genomic blot analysis: Genomic DNA was isolated from mature leaf tissue by a previously described method (SHURE, WESSLER and FEDOROFF 1983). Genomic DNA was digested with threefold excess units of restriction enzymes according to the manufacturer's recommendation (New England Biolabs or Bethesda Research Laboratories). DNA samples were electrophoresed through 0.8% agarose (Sigma) gels, and were transferred to nitrocellulose (Schleicher and Schuell) according to the method of SOUTHERN (1975). Filters were prehybridized, hybridized and washed according to previously published methods (CHOMET, WESSLER and DELLAPORTA, 1987).

Genomic cloning of *P-vv*: Maize DNA libraries were constructed with the lambda vector EMBL3 (FRISCHAUF *et al.* 1983) and genomic DNA homozygous for *P-vv* in the W22 background as previously described (S. L. DELLAPORTA 1987). Genomic DNA was digested with *Sal*I and size fractionated on glycerol gradients. This library was screened with the internal 1.6-kb *Hin*dIII fragment of *Ac9* (FEDOROFF, WESSLER and SHURE 1983). Phage DNA samples were prepared according to BERMAN *et al.* (1982). The position of the *Ac* element in the 8.0-kb *Sal*I fragment insert was determined by the location of restriction sites predicted from published *Ac* DNA sequence data (POHLMAN, FEDOROFF and MESSING 1984; MULLER-NEUMANN, YODER and STARLINGER 1985). The 2.5-kb *Sal*I-*Bgl*II DNA fragment flanking the *Ac* element was purified from agarose gels by electroelution (DRETZEN *et al.* 1981) and nick translated as described.

RESULTS

Identification of *P-vv* by genomic blot analysis: Genomic DNA was isolated from plants homozygous for the *P-vv* allele or homozygous for a colorless pericarp allele (*P-wr* or *P-ww*). Different genetic background lines including W22, W23, 4Co63 and a hybrid of W22 × W23 were analyzed. These samples were digested with *Sal*I, a restriction enzyme that does not cut within the 4.56-kb *Ac* element (POHLMAN, FEDOROFF and MESSING 1984; MULLER-NEUMAN, YODER and STARLINGER 1985), and subjected to Southern blot analysis. The *Ac* probe used in these experiments was an internal 1.6-kb *Hin*dIII fragment of *Ac9* (FEDOROFF, WESSLER and SHURE 1983) (shown in Figure 1) which is homologous to 8–10 different genomic sequences in certain inbred maize lines. These homologous sequences lack the ability to catalyze the transposition of *Ds* elements. DNA from inbreds that lack *Ac* activity showed hybridization only to the high molecular weight fraction of DNA at the top of the gel (Figure 2, lanes 1–3). The average molecular weight of this DNA (over 50 kb) suggested that this fraction of DNA was insensitive to *Sal*I digestion. To determine whether this digestion pattern by *Sal*I was complete, we digested genomic DNA mixed with λ DNA and analyzed the extent of λ digestion by reprobing the blots with nick translated λ DNA.

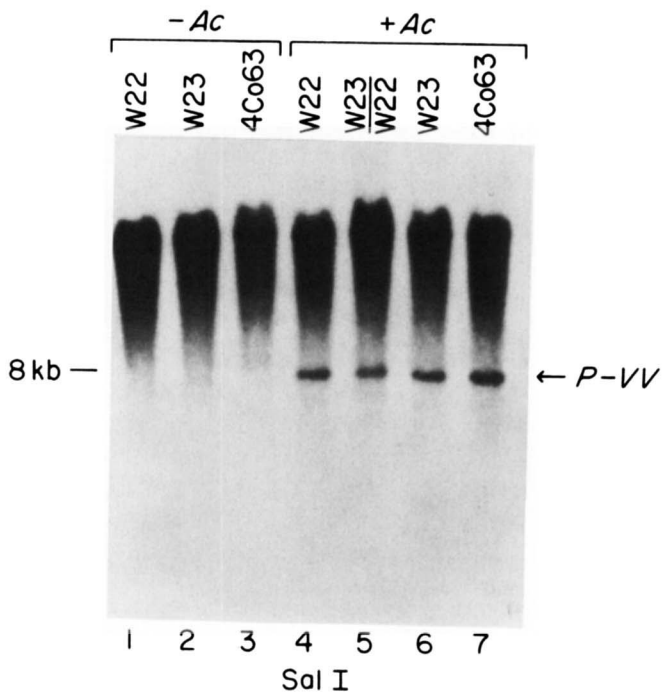

FIGURE 2.—Cosegregation of a genomic fragment with *Ac*. Genomic DNA was isolated from a plant homozygous for *P-wr* or *P-ww* (−*Ac*) or homozygous *P-vv* (+*Ac*) in the W22, W23 or 4Co63 inbred lines or a W23 × W22 hybrid line. DNA was digested with *Sal*I, Southern blotted, and probed with the *Ac* probe depicted in Figure 1. All *Ac* homologous sequences were detected in the high molecular weight region of the autoradiogram except for an 8.0-kb *Sal*I fragment found only in plants containing *Ac*.

Results of this assay indicated the digestion went to completion (data not shown). Hence, the observed pattern of hybridization is not due to incomplete digestion of the genomic DNA. Inbred lines with *Ac* at *P* contained an 8.0-kb fragment that hybridized to the *Ac* probe in addition to the hybridization in the high molecular weight DNA fraction (Figure 2, lanes 4–7). This 8.0-kb fragment was found only in plants containing the *P-vv* allele and not found in near isogenic plants without *Ac* activity (lanes 1–3). This result indicates that the 8.0-kb *Sal*I fragment may represent a genomic fragment containing the *Ac* element at the *P* locus.

Cloning the *P-vv* allele: We confirmed that the 8.0-kb band was an *Ac* containing genomic fragment of *P-vv* by cloning this band from a genomic library prepared with size-fractionated *Sal*I digested DNA. A recombinant clone hybridizing to *Ac* probe and containing an 8.0-kb *Sal*I insert was identified. A restriction map of the insert with the position of *Ac* is shown in Figure 1. The 2.5-kb *Bgl*II-*Sal*I fragment flanking *Ac* was used to probe DNA from plants carrying the *P-vv* allele and a *P-rr* allele (*P-rr:GM*) obtained by germinal excision of *Ac* from *P*. Although the *P* probe contained repetitive sequences, the 8.0-kb *Sal*I band was detected in DNA from plants containing *P-vv*

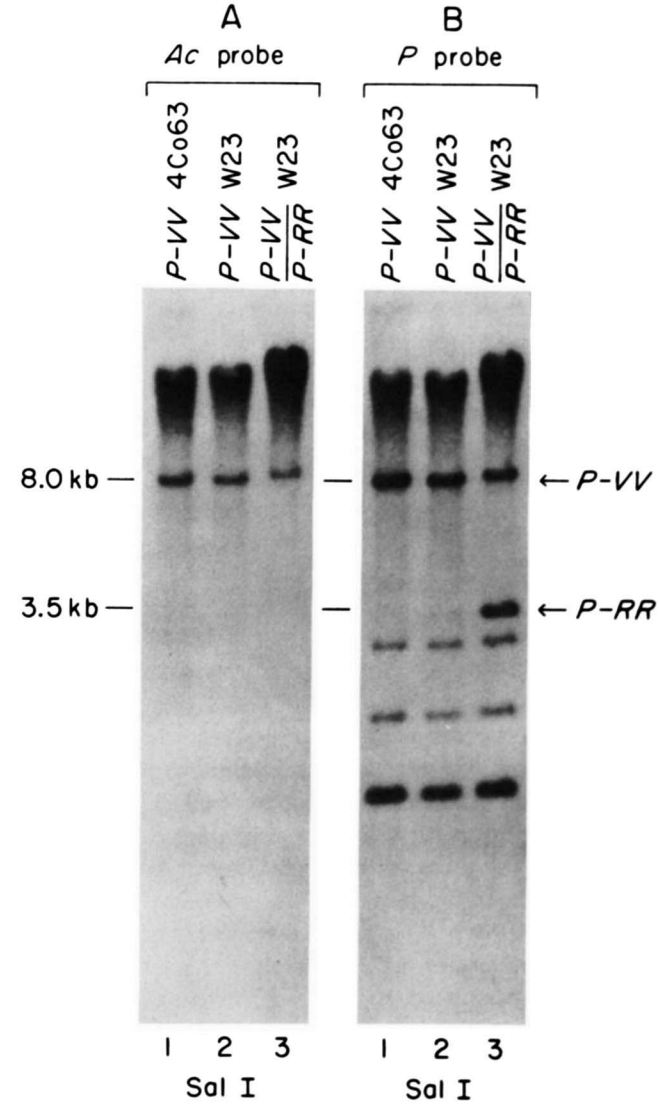

FIGURE 3.—Hybridization of *P-vv* and *P-rr:GM* DNA to *Ac* or *P* probes. (A) Genomic DNA from a plant homozygous for *P-vv* in the 4Co63 genetic background (lane 1) or W23 genetic background (lane 2) or a plant heterozygous for *P-vv/P-rr:GM* in the W23 genetic background (lane 3) were digested with *Sal*I, Southern blotted and probed with the *Ac* probe. The 8.0-kb *Sal*I fragment containing *Ac* at the *P* locus was detected in plant DNA containing *P-vv* (lanes 1–3). (B) Same DNA as in panel (A) except the blot was probed with the *P* probe depicted in Figure 1. The 8.0-kb *Sal*I fragment of *P-vv* was detected (lanes 1–3) and a 3.5-kb *Sal*I fragment in the plant containing the *P-rr:GM* allele (lane 3) which represented excision of the 4.5-kb *Ac* element from the 8.0-kb *P-vv* fragment.

(Figure 3B, lanes 1–3). DNA from the plant heterozygous for *P-vv* and *P-rr:GM* contained the 8.0-kb *Sal*I fragment and a new 3.5-kb band (lane 3). The approximate fragment size, 3.5 kb, is expected after excision of the 4.56-kb *Ac* element from the 8.0-kb *P-vv* band.

Identification of *Ac* using methylation-sensitive restriction enzymes: Since *Sal*I is a methylation-sensitive restriction enzyme (MCCLELLAND and NELSON

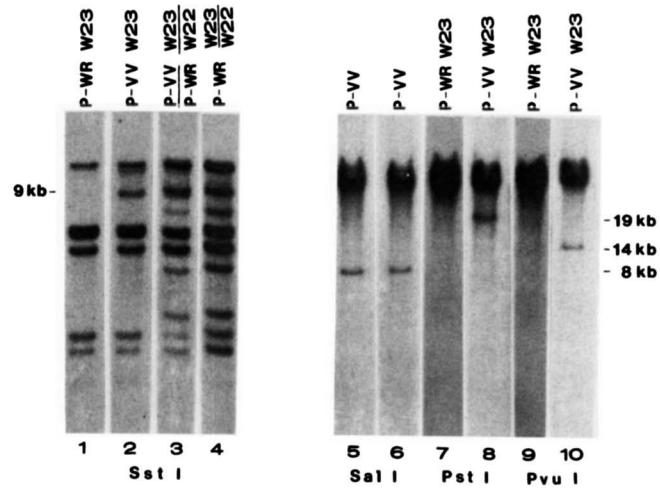

FIGURE 4.—Genomic blot analysis of inbred, hybrid, and mixed genetic lines using the *Ac* probe. DNA was isolated from inbred W23 (lanes 1, 2, 7–10), a genetic hybrid W23 × W22 (lanes 3 and 4), or a mixed genetic background (lanes 5 and 6). This DNA was digested with the methylation-insensitive restriction enzyme, *Sst*I, (lanes 1–4) or the methylation-sensitive restriction enzyme, *Sal*I (lanes 5 and 6), *Pst*I (lanes 7 and 8), or *Pvu*I (lanes 9 and 10), Southern blotted and probed with the *Ac* probe.

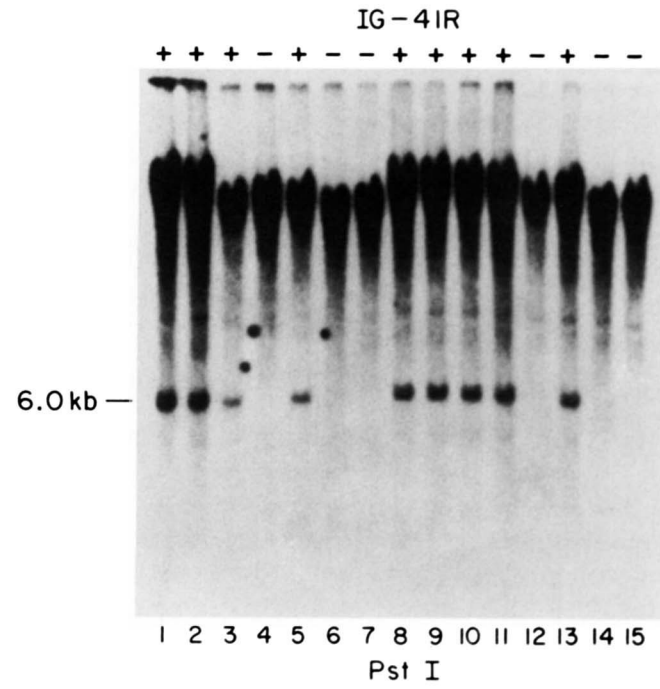

FIGURE 5.—Cosegregation of transposed *Ac* activity and a genomic fragment. Plant DNA was isolated from progeny of a red cotwin sector (IG-41R) (see GREENBLATT, 1984 for details) with *Ac* activity (+) and without *Ac* activity (−), digested with *Pst*I, Southern blotted, and probed with the *Ac* probe shown in Figure 1. *Ac* activity was detected by the ability to catalyze transposition of unlinked *Ds* element at the *R* locus. A 6.0-kb fragment was identified in DNA from plants with *Ac* activity (lanes 1–3, 5, 8–11, and 13) and not those without *Ac* (lanes 4, 6, 7, 12, 14 and 15).

1985), we asked whether *Ac* at *P* is fractionated from cryptic *Ac*-like sequences as a result of differences in the methylation of flanking DNA. Certain restriction enzymes will not cut DNA when the recognition site contains 5-methyl cytosine. Plant DNA is heavily methylated at DNA sequences containing the CG dinucleotide or the CXG trinucleotide motif, where X can be A, T, C or G (GRUENBAUM *et al.* 1981). This ability to fractionate an active *Ac* from homologous sequences in genomic DNA was important since the genetic material we analyzed was outcrossed to various inbred lines. DNA from progeny of these hybrid crosses contained 12 or more heterogenous, cryptic *Ac*-like bands when analyzed with a methylation-insensitive enzyme such as *Sst*I (Figure 4, lanes 3 and 4). However, when DNA from a mixed genetic background was analyzed with the methylation-sensitive enzyme *Sal*I, the 8.0-kb *Sal*I fragment of *P-vv* was detected in the lower molecular weight fraction of DNA, while the cryptic *Ac*-like sequences remained in the high molecular weight fraction of DNA (Figure 4, lanes 5 and 6). The restriction enzymes *Pst*I and *Pvu*I, which also contain CG and CXG sequences in their recognition sequences, gave a pattern of digestion similar to *Sal*I (Figure 4, lanes 7–10), perhaps due to the sensitivity of both enzymes to DNA methylation. In both *Pst*I and *Pvu*I digests of DNA from plants without the *P-vv* allele, hybridization was confined to the high molecular weight fraction of DNA (lanes 7 and 9). In the *Pst*I digest, a 19-kb fragment was detected in DNA of a sibling plant containing the *P-vv* allele (lane 8). In the *Pvu*I digest, a 14-kb band was detected in DNA of a sibling plant containing the *P-vv* allele (lane 10). Both the 19-kb *Pst*I fragment and the 14-kb *Pvu*I fragment hybridized to the *P* probe (data not shown). We concluded that following digestion with methylation-sensitive restriction enzymes, such as *Sal*I, *Pst*I or *Pvu*I, the active *Ac* element at *P* is fractionated from cryptic, inactive *Ac*-like sequences by differences in the degree of DNA methylation. A detailed analysis using methylation-sensitive enzymes to identify and clone active *Ac* elements will be reported elsewhere.

Molecular location of tr-*Ac* elements in cotwin mutations: Can *Ac* elements that have transposed (tr-*Ac*) from *P* be detected using methylation-sensitive restriction enzymes and genomic blot analysis? We analyzed 30 backcross progeny from a plant containing an *Ac* element that had transposed from the *P-vv* allele using the methylation-sensitive enzyme *Pst*I.

This plant originated from a red cotwin sector in a W23 × 4Co63 hybrid background and contained a transposed *Ac* element unlinked to *P* (GREENBLATT 1984). A representative sample of these genomic blots is shown in Figure 5. The data indicated a 6.0-kb *Pst*I fragment that hybridized to the *Ac* probe was segregating in this population of plants. These progeny were pollinated with a *Ds*-suppressed *R-sc* allele to test for *Ac* activity. Every plant containing this 6.0-kb *Pst*I

fragment yielded an ear showing red or colorless pericarp with their kernel progeny showing 1:1 ratio for variegated:colorless aleurone (data not shown). Such results are expected when the female parent contains one *Ac* element unlinked to *P*. Plants that did not contain the 6.0-kb *Pst*I fragment gave ears with red or colorless pericarp and all colorless kernel progeny (no *Ac* activity). These results indicate that the 6.0-kb *Pst*I fragment cosegregated with *Ac* activity and was segregated randomly with respect to the *P* locus.

Using methylation-sensitive restriction enzymes to follow tr-*Ac* elements, we asked whether a cotwin sector represents mitotic descendants from a single daughter cell involved in *Ac* transposition. Since a chromosome carrying a tr-*Ac* element from a somatic pericarp sector will segregate 1:1 in the meiotic division necessary for formation of the underlying female gametophyte, half of the progeny should have the same genomic fragment containing the tr-*Ac*. We analyzed progeny from a twin sector in which the red sector was comprised of 10 underlying kernels. Four plants derived from the red sector had *Ac* activity based on the *Ds* test (data not shown) and the DNA from these plants contained the same 6.0-kb *Pst*I fragment representing the tr-*Ac* element (Figure 6, lanes 1–4). Thus, the sector represents clonal divisions of daughter cells from a single transposition of *Ac* from the *P* locus.

Based on previous genetic analysis of twin mutations (GREENBLATT and BRINK 1962; GREENBLATT 1968, 1974, 1984), the current model for *Ac* transposition from the *P* locus predicts a transposed *Ac* element should be present at identical chromosomal positions in both red and light-variegated sectors of type I cotwins. We tested this hypothesis by examining tr-*Ac* elements found in progeny from individual red and light-variegated twinned mutations. DNA was isolated from plants of twin origin in which the tr-*Ac* in the light-variegated sector was mapped distal, proximal, or unlinked to *P* (GREENBLATT 1984). The position of the tr-*Ac* in the red sector of each twin was not mapped. This DNA was digested with a methylation-sensitive restriction enzyme and subjected to genomic blot analysis. In the progeny of each twin, we detected a fragment that hybridized to *Ac* probe and which cosegregated with the transposed *Ac* activity. Representative blots of instances in which the transposed *Ac* element was located distal, proximal or unlinked to the *P* locus are shown in Figures 7, A and B; 8, A and B; and 9, respectively. Since the tr-*Ac* element was located on the same genomic restriction fragment in red and light-variegated progeny from a single twin event, we concluded that the tr-*Ac* element was at the same genomic position in red and light-variegated

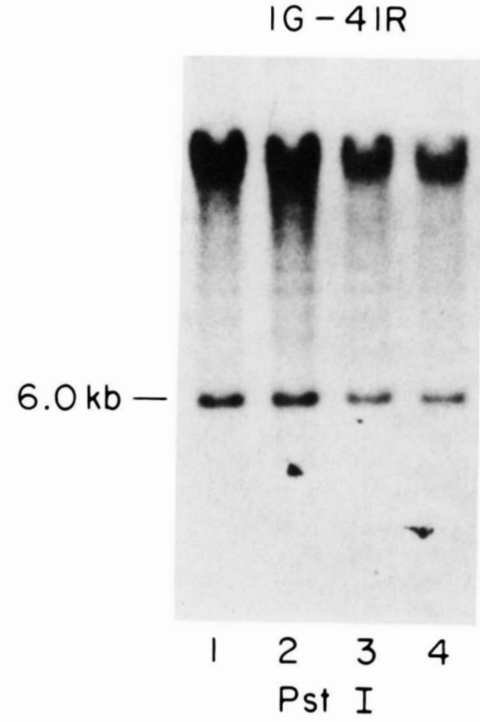

FIGURE 6.—A red sector is derived from a single somatic clone. DNA was isolated from plants with *Ac* activity and red pericarp ears, originating from individual kernels of a red cotwin sector (IG-41R) (GREENBLATT 1984), digested with *Pst*I, Southern blotted and probed with the *Ac* probe. In every sample the tr-*Ac* element was located in the same 6.0-kb *Pst*I fragment, indicating that the kernels under the red sector were from mitotic descendants of a daughter cell derived from a single somatic transposition of *Ac* from the *P* locus.

lineages. This was found to be true regardless of the genetic position of the tr-*Ac* element in relation to the *P* locus. However, as expected, among progeny from different twinned mutations, the tr-*Ac* element was located on different genomic fragments (compare Figures 7, A and B; 8, A and B; and 9). To eliminate the possibility of fortituous comigration of the *Ac*-hybridizing fragment in light-variegated and red sector progeny, we also digested each DNA with a second methylation-sensitive restriction enzyme, *Pvu*I. Examples of these blots are shown in Figures 7C and 8C. These results also show comigration of a band that hybridized to the *Ac* probe in both light-variegated and red sector progeny.

DISCUSSION

We have taken a molecular approach to analyze *Ac* transposition from the *P* locus of maize. Because of the repetitiveness of *Ac* DNA (FEDOROFF, WESSLER and SHURE 1983), it was necessary to define conditions which would distinguish the active *Ac* element from homologous, inactive sequences independent of the chromosomal position of *Ac*. Genomic blot analysis of maize DNA using methylation-sensitive restriction en-

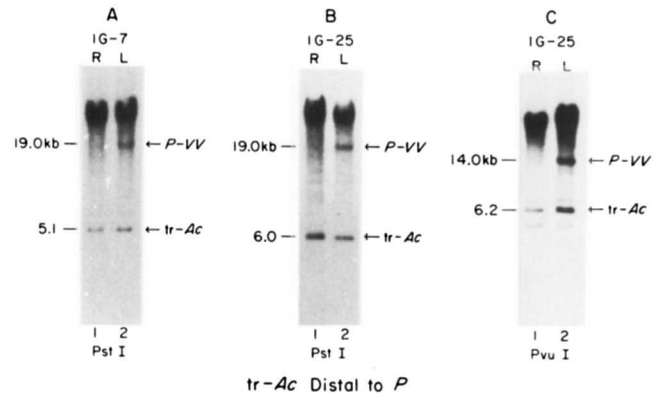

FIGURE 7.—Analysis of the tr-*Ac* element located distal to the *P* locus in progeny from twin mutations. (A) Genomic DNA was isolated from progeny from a red (R) or light-variegated (L) cotwin sector that contained tr-*Ac* activity (IG-7) (GREENBLATT 1984), digested with *Pst*I, Southern blotted and probed with the *Ac* probe. The genomic fragment containing the tr-*Ac* element was an identical size (5.1 kb) in DNA from both the red (lane 1) and light-variegated progeny (lane 2). The 19-kb *Pst*I fragment representing the *Ac* at *P-vv* was detected only in the DNA from the light-variegated progeny (lane 2). (B) Analysis of a second twin (IG-25) (GREENBLATT, 1984) as in (A). The tr-*Ac* was located on a 6.0-kb *Pst*I fragment in both the red (lane 1) and light-variegated (lane 2) progeny. The 19-kb *Pst*I fragment of *P-vv* was detected only in the DNA from light-variegated progeny (lane 2). (C) Same as in (B) except the genomic DNA was digested with *Pvu*I. The tr-*Ac* element was detected on a 6.2-kb *Pvu*I fragment in both red (lane 1) and light-variegated (lane 2) progeny. The 14-kb *Pvu*I fragment represents *Ac* at *P* was found only in the light-variegated progeny (lane 2). The weakly hybridizing bands seen in lanes 1 and 2 were also detected in DNA of siblings without the tr-*Ac* (data not shown) and do not represent fragments with *Ac* activity.

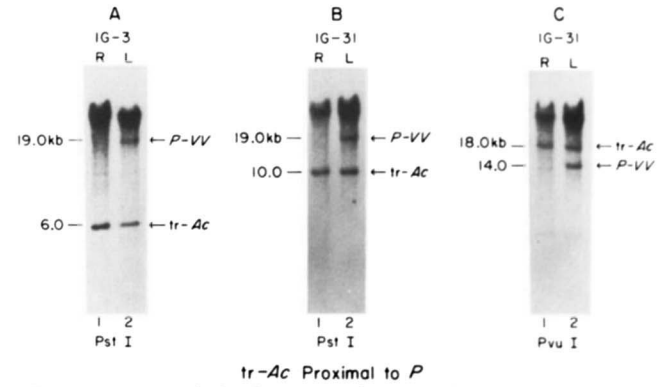

FIGURE 8.—Analysis of progeny from a twin mutation containing a tr-*Ac* element located proximal to the *P* locus. (A) Genomic DNA was isolated from red (R) or light-variegated (L) progeny from a twin mutation (IG-3) (GREENBLATT 1984), digested with *Pst*I, Southern blotted and probed with the *Ac* probe. Both the red (lane 1) and light-variegated (lane 2) progeny contained a 6.0-kb *Pst*I fragment that represented the tr-*Ac*. The light-variegated progeny also contained the 19-kb *Pst*I fragment of *P-vv*. (B) Same analysis as in (A) except from a different twin mutation (IG-31) (GREENBLATT 1984). The tr-*Ac* in both red (lane 1) and light-variegated (lane 2) progeny was located on a 10-kb *Pst*I fragment. The 19-kb *Pst*I fragment of *P-vv* was detected only in the light-variegated progeny. (C) Same analysis as in (B) except genomic DNA was digested with *Pvu*I. The tr-*Ac* fragment was detected in both red (lane 1) and light-variegated (lane 2) progeny as an 18-kb *Pvu*I fragment. The 14-kb *Pvu*I fragment of *P-vv* was detected only in the light-variegated progeny.

zymes has shown that the inactive sequences are usually hypermethylated while active *Ac* elements are hypomethylated (DELLAPORTA and CHOMET 1985; CHOMET, WESSLER and DELLAPORTA 1987). In this paper, we have shown that the DNA immediately flanking active *Ac* elements appears to be hypomethylated. This distinction allows fractionation of the active *Ac* element from cryptic, inactive sequences. If integrations of *Ac* were randomly distributed, this result would be unlikely since over 80% of plant DNA is methylated (GRUENBAUM *et al.* 1981). It is possible that *Ac* elements prefer target sites in regions of hypomethylation. Such a preference would result if the transposase is methylation-sensitive or sensitive to some type of secondary structure in DNA correlated with methylation patterns. Alternatively, integration of an active, hypomethylated element may have a spreading effect and result in the loss of methylation in flanking DNA sequences. To distinguish between these possibilities, the region flanking receptor sites would need to be examined before and after integration of an active *Ac* element.

These findings have a practical application. *Ac* can cause mutations by insertion into genes and these mutant alleles can be cloned with *Ac* probes (FEDOROFF, FURTEK and NELSON 1984). In mixed genetic lines, it becomes increasingly difficult to distinguish *Ac* from cryptic sequences as the number of the cryptic sequences approaches 15 or more fragments (J. CHEN, unpublished results). However, it is possible to rapidly locate transposed *Ac* elements using methylation-sensitive restriction enzymes such as *Sal*I and *Pst*I for gene cloning purposes. We have demonstrated this strategy is possible by cloning a molecular probe of the *P-vv* allele using an *Ac* probe.

Our analysis of type I mutations indicates that transposed *Ac* elements are located on identical restriction fragment in both red and light-variegated lineages of the same twin and are on different fragments in different twin mutations. It is unlikely that this comigration is fortuitous. However, to test this possibility we repeated this analysis using a different methylation-sensitive restriction enzyme. In each case both red and light-variegated progeny contained a comigrating *Ac*-homologous fragment. We interpret these results to mean that the transposed *Ac* element in type I cotwins integrated before replication of the receptor site was completed. However, the donor site was replicated before excision of *Ac* since the red daughter lineage lost *Ac* at *P* while the light-variegated lineage retained *Ac* at *P*.

This mechanism for *Ac* transposition would always lead to potential twin formation (red and light-variegated daughter lineages) if the transposed *Ac* was

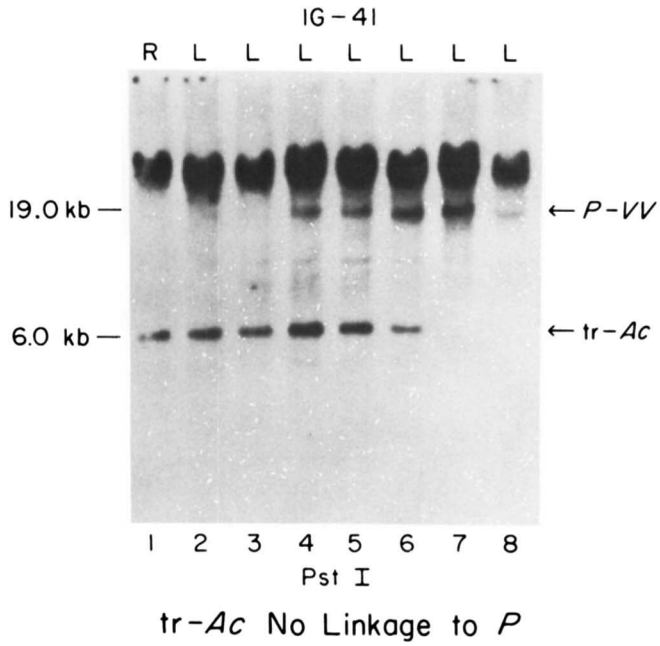

FIGURE 9.—Analysis of a twin mutation containing a tr-*Ac* element unlinked to the *P* locus. Genomic DNA was isolated from red (R) and light-variegated (L) progeny containing *Ac* activity of a twin mutation (IG-41) (GREENBLATT 1984) where the tr-*Ac* activity was linked to the *P* locus, digested with *Pst*I, Southern blotted and probed with the *Ac* probe. A 6.0-kb *Pst*I fragment was detected in both the red (lane 1) and light-variegated (lanes 2–6) progeny containing tr-*Ac* activity. Light-variegated progeny without tr-*Ac* activity that gave medium variegated ears only contained the 19-kb *Pst*I fragment of *P-vv* (lanes 7 and 8). Light-variegated progeny that gave colorless pericarp ears contained only the 6.0-kb *Pst*I fragment (lanes 2 and 3), while light-variegated progeny that gave light-variegated ears contained both the 6.0-kb and 19-kb *Pst*I fragments (lanes 4–6).

always present in the cell which receives *P-vv*. The phenotype of the sister cell that lost *Ac* from *P* is red and independent of the presence of an additional *Ac*. Do all transpositions of *Ac* from the *P* locus result in twinned mutations? Certainly, untwinned red or light-variegated sectors do appear on medium-variegated ears. Several explanations for untwinned mutations are possible. It is possible that these events represent an alternate mechanism for *Ac* transposition that does not require replication of *Ac* before excision. However, an alternative explanation for such results is that the planes of cell divisions that give rise to the ear could result in the loss of one of the cotwin lineage to the final pericarp tissue. Assuming both lineages can be lost at an equal probability, the absolute number of red or light-variegated sectors that appear untwinned should be equal if all transpositions result in twin mutations. Genetic evidence that untwinned red and light-variegated sectors are in fact equal in number (GREENBLATT 1974) supports the hypothesis that *Ac* transposes only after replication. This mechanism is not found in the replicative and conservative type mechanisms described for transposition of prokaryotic elements such as Tn*10* and Mu (reviewed by DERBYSHIRE and GRINDLEY 1986).

We have not yet completed a detailed analysis of progeny from type II cotwins. These events are distinguished from type I events in that no active *Ac* element is found in progeny from cotwin red sectors. Our preliminary analysis has indicated that the transposed *Ac* that was found in the light-variegated progeny was not detected by genomic blot analysis in the red cotwin progeny (J. CHEN, I. M. GREENBLATT and S. L. DELLAPORTA, unpublished results).

We wish to thank TERRY ASHLEY, PAUL CHOMET, BRENDA LOWE, TIMOTHY NELSON, and JANE LANGDALE for their helpful discussions and critical reading of the manuscript and LORRAINE KLUMP for word processing. This work was supported by a National Institute of Health grant (R01-GM38148-01) to S.L.D.

LITERATURE CITED

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. USA **40:** 1118–1126.

BERMAN, M. L., L. W. ENQUIST and T. J. SILHAVY, 1982 *Advanced Bacterial Genetics.* Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

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

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.

DELLAPORTA, S. L. and P. S. CHOMET, 1985 The activation of maize controlling elements. pp. 169–216. In: *Genetic Flux in Plants*, Edited by B. HOHN and E. S. DENNIS. Springer-Verlag, New York.

DERBYSHIRE, K. M. and N. D. F. GRINDLEY, 1986 Replicative and conservative transposition in bacteria. Cell **47:** 325–327.

DRETZEN, G., M. BELLARD, P. SASSONE-CORSI and P. CHAMBON, 1981 A reliable method for the recovery of DNA fragments from agarose and acrylamide gels. Anal. Biochem. **112:** 295–301.

EMERSON, R. A., 1914 The inheritance of a recurring somatic variation in variegated ears of maize. Am. Nat. **48:** 87–115.

FEDOROFF, N. V., D. B. FURTEK and O. E. NELSON, JR., 1984 Cloning of the *bronze* locus in maize by a simple and generalizable procedure using the transposable controlling element *Ac*. Proc. Natl. Acad. Sci. USA **81:** 3825–3829.

FEDOROFF, N., S. WESSLER and M. SHURE, 1983 Isolation of the transposable maize controlling elements *Ac* and *Ds*. Cell **35:** 235–242.

FRISCHAUF, A. M., H. LEHRACH, A. M. POUSTKA and N. MURRAY, 1983 Lambda replacement vectors carrying polylinker sequences. J. Mol. Biol. **170:** 827–842.

GREENBLATT, I. M., 1966 Transposition and replication of *Modulator* in maize. Genetics **53:** 361–369.

GREENBLATT, I. M., 1968 The mechanisms of *Modulator* transposition in maize. Genetics **58:** 558–597.

GREENBLATT, I. M., 1974 Movement of *modulator* in maize: a test of a hypothesis. Genetics **77:** 671–678.

GREENBLATT, I. M., 1984 A chromosomal replication pattern deduced from pericarp phenotypes resulting from movements

of the transposable element, *modulator*, in maize. Genetics **108:** 471–485.

GREENBLATT, I. M. and R. A. BRINK, 1962 Twin mutations in medium variegated pericarp maize. Genetics **47:** 489–501.

GRUENBAUM, Y., T. NAVEH-MANY, H. CEDAR and A. RAZIN, 1981 Sequence specificity of methylation in higher plant DNA. Nature **292:** 860–862.

MCCLELLAND, M. and M. NELSON, 1985 The effect of site specific methylation on restriction endonuclease digestion. Nucleic Acids Res. **13:** r201–r207.

MULLER-NEUMAN, M., J. I. YODER and P. STARLINGER, 1985 The DNA sequence of the transposable element *Ac* of *Zea mays*. Mol. Gen. Genet. **198:** 19–24.

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

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.

Communicating editor: S. D. TANKSLEY