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Transposition of Reversed *Ac* Element Ends Generates Chromosome Rearrangements in Maize

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ABSTRACT

In classical "cut-and-paste" transposition, transposons are excised from donor sites and inserted at new locations. We have identified an alternative pathway in which transposition involves the 5′ end of an intact *Ac* element and the 3′ end of a nearby terminally deleted *fAc* (*fractured Ac*). The *Ac* and *fAc* elements are inserted at the maize *p1* locus on chromosome 1s in the same orientation; the adjacent ends of the separate elements are thus in reversed orientation with respect to each other and are separated by a distance of ~13 kb. Transposition involving the two ends in reversed orientation generates inversions, deletions, and a novel type of local rearrangement. The rearrangement breakpoints are bounded by the characteristic footprint or target site duplications typical of *Ac* transposition reactions. These results demonstrate a new intramolecular transposition mechanism by which transposons can greatly impact genome evolution.

CONVENTIONALLY, a transposable element is considered to be a linear DNA segment that can move from one molecular location to another. Transposition of type II (DNA) elements is thought to occur via two general mechanisms: in replicative transposition, the transposon is copied into a new site without excision from the donor site; whereas, in the "cut-and-paste" mechanism, the transposon is excised from the donor site and inserted at the target site (Craig 2002). The molecular mechanism of cut-and-paste transposition has been elucidated for certain prokaryotic transposons such as Tn5, Tn7, and Tn10 (Craig 2002; Haniford 2002; Reznikoff 2002), but is largely unknown for eukaryotic transposable elements (TEs). However, it is commonly thought that transposition is initiated by binding of the element-encoded transposase to the terminal sequences at the 5′ and 3′ ends of the transposon, followed by endonucleolytic cleavage at the junctions between the transposon and the flanking genomic DNA. The excised transposon ends can then be inserted at a new site in the genome. From a biochemical point of view, the pair of 5′ and 3′ transposon termini are substrates of the transposase protein. Eukaryotic genomes commonly contain many copies of each type of transposon, and thus the terminal sequences are often present in multiple copies. Theoretically, a pair of 5′ and 3′ termini from different transposon copies could participate in a transposition reaction. This type of unconventional transposition (UT) event could generate a variety of chromosomal rearrangements and thereby play an important role in genome evolution.

In Drosophila, activity of *P* transposable elements is associated with recombination in males; this has been attributed to UT events involving a pair of *P*-element termini on sister chromatids or homologous chromosomes. The presence of an intact mobile *P* element in one homologous chromosome results in a male recombination frequency of ~0.5–1%; this is caused by sister chromatid transposition (SCT), *i.e.*, by transposition reactions involving *P*-element 5′ and 3′ ends on different sister chromatids (Preston *et al.* 1996). However, the presence of a 5′- and 3′-terminally deleted *P* element on each copy of homologous chromosomes results in a male recombination frequency of ~30%; this effect is caused by interchromosome transposition events (Gray *et al.* 1996).

In maize, the *Ac/Ds* transposable element system comprises the autonomous element *Activator* and the nonautonomous element *Dissociation*. The *Ac/Ds* system was first recognized by virtue of its ability to cause chromosome breakage (McClintock 1947). The *Ds* elements that produce a high frequency of chromosome breakage are termed state I, while nonbreaking *Ds* elements are termed state II. The best-studied state I *Ds* element is known as *double Ds*; it contains two copies of a simple (state II) *Ds* element, with one *Ds* element inserted into the other *Ds* element in opposite orientation (Doring *et al.* 1984). Two adjacent (state II) *Ds* elements in opposite orientation can also cause chromosome breakage in the presence of *Ac* (Weil and Wessler 1993), as can a pair of 5′ and 3′ *Ds* termini in direct orientation (English *et al.* 1993). PCR and sequencing analysis showed that chromosome breakage is caused by SCT

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events involving a pair of 5′ and 3′ ends of *Ds* elements on sister chromatids (Weil and Wessler 1993; English *et al.* 1995). In addition, Dooner and Belachew (1991) showed that closely linked *Ac/Ds* elements can also induce chromosome breakage.

To further characterize the molecular substrates of UT reactions, we studied various *Ac/Ds* insertion alleles of the maize *p1* gene. The *p1* gene encodes a Myb-homologous transcription factor that regulates the synthesis of red flavonoid pigments in various floral organs including kernel pericarp and cob (Grotewold *et al.* 1991, 1994). Hence, structural alterations in the *p1* gene caused by transposition reactions can be easily identified and studied. We isolated an allele termed the *p1-vv9D9A* allele, which contains a whole *Ac* element and a fractured *Ac* (*fAc*; 2526 bp 5′ portion is deleted) inserted in intron 2 of the *p1* gene. From *p1-vv9D9A*, we isolated two derivative alleles (one a deletion, the other a duplication) from a twinned pericarp sector. Sequences of the rearrangement junctions proved that these alleles were the reciprocal products of a single transposition reaction involving a pair of *Ac* 5′ end and *fAc* 3′ ends from different sister chromatids (Zhang and Peterson 1999).

Although unconventional transposition events have been the subject of much research, transposition events involving TE termini in reversed orientation have not yet been described in a eukaryote. To test whether such "reversed-ends" transposition could occur, we studied a maize *p1* allele that carries a pair of *Ac* termini in reversed orientation. We show that this configuration of transposon ends can generate deletions, inversions, and a novel local rearrangement structure and that the breakpoints of these rearrangements are delineated by the sequence hallmarks of *Ac/Ds* transposition. We discuss the possible role of this transposition mechanism in mediating the various types of chromosomal rearrangements that have shaped eukaryotic genomes.

MATERIALS AND METHODS

Genetic stocks: Alleles of the *p1* gene are identified by a two-letter suffix that indicates their expression pattern in pericarp and cob: *e.g.*, *P1-rr* (red pericarp and red cob); *P1-wr* (white pericarp, red cob); and *p1-ww* (white pericarp and white cob). The standard *p1-vv* (variegated pericarp and variegated cob) allele described by Emerson (1917) contains an *Ac* insertion in the second intron of a *P1-rr* gene. From *p1-vv*, we obtained a spontaneous derivative termed *P1-ovov1114* (orange-variegated pericarp and orange-variegated cob), in which the *Ac* element had undergone an intragenic transposition to a site 153 bp upstream in *p1* gene intron 2 and inserted in the opposite orientation (Peterson 1990). From *P1-ovov1114*, we obtained a spontaneous derivative termed *p1-vv9D9A* (Zhang and Peterson 1999); this allele contains an *Ac* element, a 112-bp rearranged *p1* gene fragment (rP), and the terminally deleted *Ac* element *fAc*. From *p1-vv9D9A*, we obtained the allele *P1-rr11* described here; this allele was generated from *p1-vv9D9A* by excision and transposition of the full-length *Ac* element from its location in intron 2 of *p1* to a site ~9 kb upstream of the *p1* transcription start site (Figure 1).

TABLE 1

Oligonucleotide primers

Primer	Sequence
p1-1	TGTTCCTTCTGCCCTGAGTCCTG
Ac-2	ATTTTACCGACCGTTACCGACC
Ac-3	TTATCCCGTTCGTTTTCGTTACC
Ac-4	CCCGTTTCCGTTCCGTTTTCGT
Ac-5	TACGATAACGGTCGGTACGGG
p1-6	GACAGTTCGCAGTTGGGTTGGG
p1-7	TCCGTCCTCAAAACCAAAGCG
p1-8	AGAGGAATACCTTAGACTTGG
p1-9	GAAAGGTTGTGGAGAATAATAATAAGTAGGGCA
p1-10	AACTGCAGGGCAACACTAGGCACAACGAC
p1-11	GATGATGTCTTCTTCCTCCTTGG
p1-12	TAGATTTCCGTTCTTCGTGTGA
p1-13	CTGGCGAGCTATCAAACAGGCCAC
p1-14	ACATTGAACTGGGATTGTCTGCTTTG
p1-15	GGTTTTGAGGACGGAGGAGG

Genomic DNA extractions and Southern blot hybridization: Total genomic DNA was prepared from husk using a modified cetyltrimethylammonium bromide extraction protocol (Saghai-Maroof *et al.* 1984). Agarose gel electrophoresis and Southern hybridizations were performed as described (Sambrook *et al.* 1989), except hybridization buffers contained 250 mm $NaHPO_4$, pH 7.2, 7% SDS, and wash buffers contained 20 mm $NaHPO_4$, pH 7.2, 1% SDS.

PCR amplifications: PCR amplifications were performed as described (Saiki 1989) using the oligonucleotide primers shown in Table 1.

Reactions were heated at 94° for 3 min and then cycled 35 times at 94° for 20 sec, 60° for 30 sec, and 72° for 1 min/1 kb length of expected PCR product and then at 72° for 8 min. The band amplified was purified from an agarose gel and sequenced directly. Sequencing was done by the DNA Synthesis and Sequencing Facility, Iowa State University.

RESULTS

The *P1-rr11* allele and its derivatives: We isolated a *p1* allele designated *P1-rr11* (*r*ed pericarp, *r*ed cob), which contains the truncated *Ac* element *fAc*, inserted in the second intron of *p1*, and a full-length *Ac* element, inserted in the *p1* 5′ flanking sequences, 13,175 bp upstream of the *fAc* element (materials and methods). In *P1-rr11*, the 5′ end of *Ac* and the 3′ end of *fAc* are oriented toward each other (Figure 1A). *P1-rr11* exhibits unstable kernel pigmentation, as evidenced by frequent colorless pericarp sectors and colorless kernels (Figure 1B). Because the kernel pericarp and egg cell are derived from a common cell lineage, sporophytic mutations that give rise to sectors of colorless pericarp can be germinally transmitted through the kernel embryo (Greenblatt 1985). Colorless and light-colored kernels were selected from 20 ears of genotype *P1-rr11/P1-wr*, and from these we obtained 10 new *p1-ww* (*w*hite

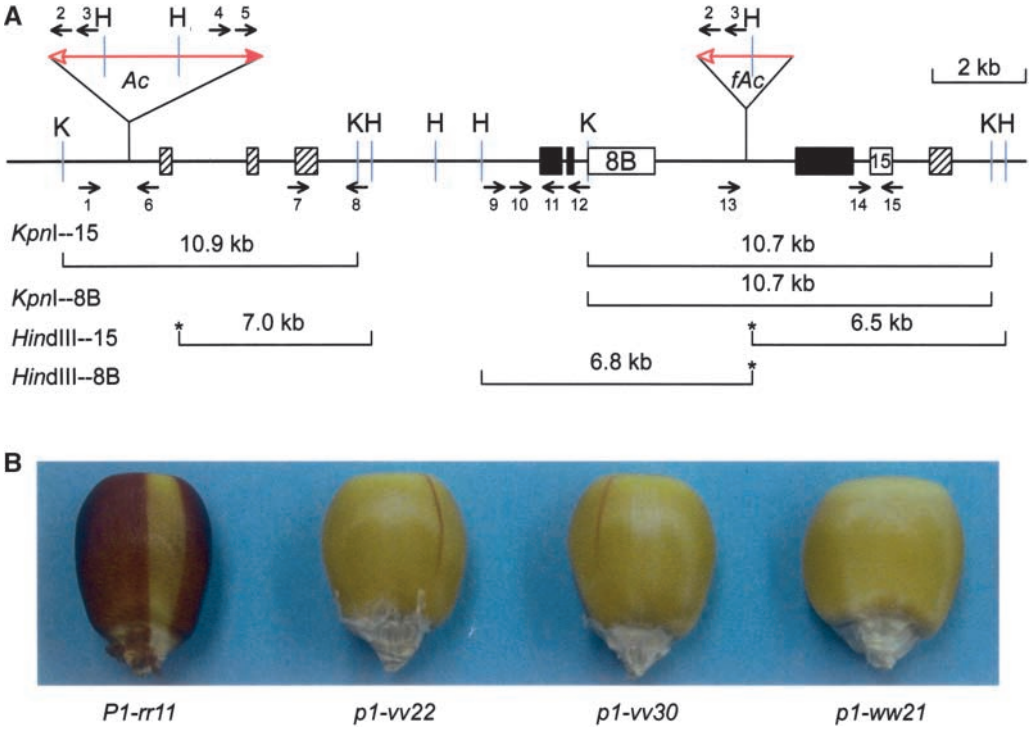

FIGURE 1.—Gene structure and phenotypes of *P1-rr11* and derivative alleles. (A) Restriction map of the *p1* gene and surrounding sequences in the *P1-rr11* allele. The solid boxes are *p1* gene exons 1, 2, and 3 (left to right). Red triangles indicate *Ac* or *fAc* insertions, and the open and solid arrowheads indicate the 3′ and 5′ ends, respectively, of *Ac*/*fAc*. The positions of fragments 8B and 15 used as probes are indicated by the numbered boxes. Additional sequences homologous to 15 are indicated as hatched boxes. The short horizontal arrows indicate the orientations and approximate position of PCR primers. Primers are identified by numbers above or below the arrows; primer prefixes used in the text (p1, Ac) are omitted due to space constraints. K, *Kpn*I; H, *Hin*dIII. Lines below the map indicate the restriction fragments produced by digestion with *Kpn*I or *Hin*dIII and hybridizing with the indicated probe; asterisks indicate *Hin*dIII restriction sites located within *Ac* or *fAc* sequences. (B) The kernel pigmentation phenotype of *P1-rr11* and its derivatives. Typical representative kernels are shown for *P1-rr11* and *p1-ww21*. For *p1-vv22* and *p1-vv30*, most kernels have colorless pericarp; only a small fraction of kernels have red sectors as shown here. Kernels shown are from plants in which the indicated allele is heterozygous with a standard recessive *p1-ww* allele for colorless pericarp and cob.

pericarp, *w*hite cob) alleles and two new *p1-vv* (*v*ariegated pericarp, *v*ariegated cob) alleles (Figure 1B).

The structures of the alleles derived from *P1-rr11* were analyzed by Southern blot hybridization using *p1* genomic fragments 15 and 8B as probes (Figure 2). Probe 15 detects an enhancer-associated fragment and related sequences located at several sites upstream and downstream of the *p1* gene (Figure 1A), whereas probe 8B detects the *p1* coding sequence and also cross-hybridizes with the coding sequence of a linked paralagous gene (*p2*) that is located >20 kb upstream of the *p1* gene (ZHANG *et al.* 2000, 2003). The results of the *Kpn*I-15 blot shows that significant rearrangements occurred in all the alleles: while the progenitor allele *P1-rr11* gives a doublet band of 10.9 and 10.7 kb, the 12 derivative alleles show a different size band or no hybridization with the probe. Moreover, the *Kpn*I-8B, *Hin*dIII-15, and *Hin*dIII-8B blots show that *p1* sequences located between the *Ac* and *fAc* termini have been deleted in the 10 new *p1-ww* alleles, while in the 2 *p1-vv* alleles (*p1-vv22* and *p1-vv30*) the probe detects bands of altered size (see *Generation of local rearrangements*). In contrast, bands derived from the linked *p2* gene are not altered in the derivative alleles with the exception of the allele *p1-ww25* (see *Generation of deletions*).

Reversed *Ac* end transposition model: The high frequency of *p1* sequence deletion observed is consistent with a model for transposition reactions involving *Ac* ends in reversed orientation (Figure 3; see animation available at http://www.genetics.org/supplemental/). In the case of *P1-rr11*, the 5′ end of the intact *Ac* element and the 3′ end of the *fAc* element are transposition substrates (Figure 3A). Transposon excision generally is accompanied by rejoining of the flanking host DNA; in *P1-rr11*, joining of the sites flanking the 5′ end of the intact *Ac* element and the 3′ end of the *fAc* element would form a 13-kb circle (Figure 3B). This type of small extrachromosomal circle would ordinarily be lost, resulting in deletion of *p1* sequences including fragment 8B, as was observed for the 10 *p1-ww* alleles by genomic Southern blot analysis (Figure 2). However, if the transposon ends inserted into a site in the 13-kb circle, a linear chromosome would be regenerated and the *p1* sequences between the reversed *Ac* termini would be rearranged (Figure 3C), whereas insertion of the transposon ends into sites in the flanking DNA would generate either inversions (Figure 3D) or deletions (Figure 3E), depending on the orientation with which insertion into the target site occurs.

It is important to distinguish whether chromosomal rearrangements originate from transposition *per se* or from other host-encoded repair and/or recombination mechanisms. The reversed-ends transposition model predicts that rearrangement breakpoints should be

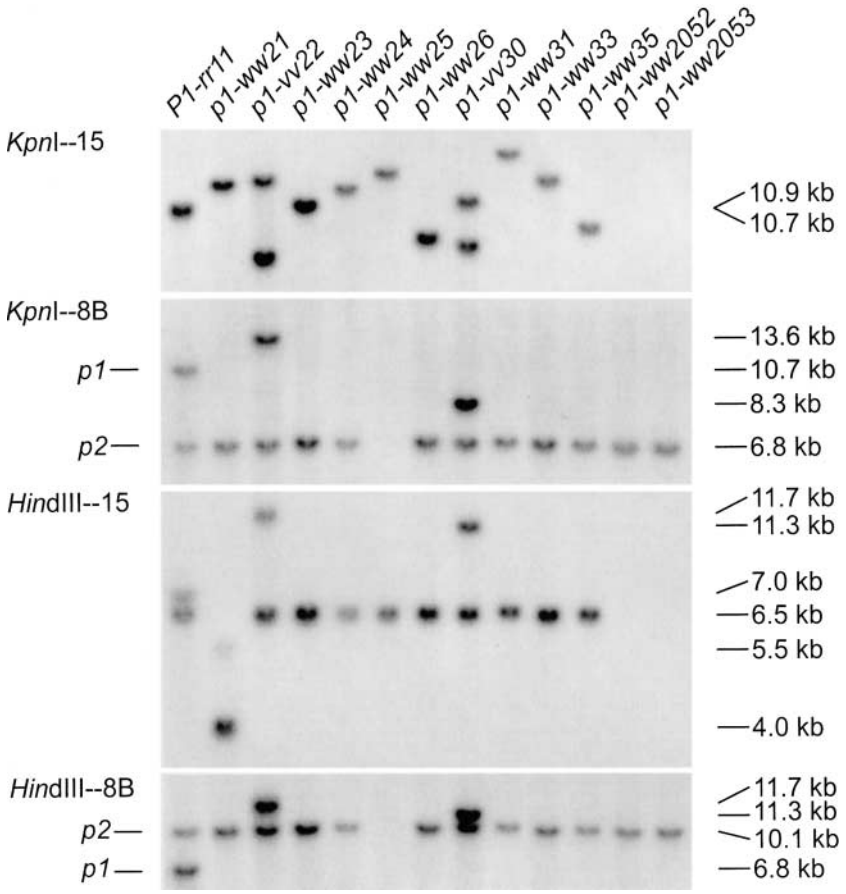

FIGURE 2.—Genomic Southern blot analysis of *P1-rr11* and its derivatives. Genomic DNA was prepared from plants homozygous for the indicated alleles, cut with *Kpn*I and *Hin*dIII, and hybridized with probes 15 and 8B from the *p1* gene. In the *Kpn*I-15 blot, the band in *P1-rr11* is a doublet that contains fragments derived from the probe 15-homologous sequences located 5′ and 3′ of the *p1* gene (Figure 1). The common band in *Kpn*I-8B and *Hin*dIII-8B blots arises from cross-hybridization with a 8B-homologous sequence in the *p2* gene (ZHANG *et al.* 2000).

bounded by transposon footprint and target site duplications (TSD) that are characteristic of transposase-mediated events. Ideally, the sequences of the footprint and TSD should be identified for the products of a single transposition reaction. The deletions are not useful for this purpose because they carry only one TSD sequence [the other TSD sequence is associated with the circularized segment that would most likely be lost (Figure 3E)], whereas the inversions contain both TSD sequences, but no footprints (Figure 3D). In contrast, a local rearrangement produced by insertion of the transposon ends into the 13-kb excised circle would contain both the TSDs and the transposon footprint generated in a single transposition event (Figure 3C). In the following sections we present the evidence for the formation of each of these products through transposition of reversed *Ac* termini.

Generation of local rearrangements: Genomic Southern blot analysis of the *p1-vv22* and *p1-vv30* alleles derived from *P1-rr11* indicates that the *p1* probe sequences are rearranged, but not deleted, in these alleles (Figure 2). According to the reverse-ends transposition model, excision of transposon ends would be accompanied by joining together the ~13 kb of genomic sequences between the reversed 5′ and 3′ *Ac* termini (Figure 3B). The new junction should contain a characteristic *Ac* footprint. Therefore, we PCR amplified genomic DNA from *P1-rr11* and derivative alleles using primers p1-6 and p1-13, which are complementary to *p1* sequences flanking the *Ac* 5′ and the *fAc* 3′ termini (see Figure 1A for the orientations and approximate positions of the PCR primers). Importantly, the primers are in divergent orientations in the progenitor allele *P1-rr11*. As expected, no PCR products were obtained from *P1-rr11* or *p1-ww* alleles containing *p1* deletions; however, *p1-vv22* and *p1-vv30* produced PCR products of the correct size (510 bp). Direct sequencing of the PCR products showed that the genomic sequences flanking the *Ac* 5′ and 3′ termini in *P1-rr11* were indeed joined together as predicted and that both junctions contained typical *Ac* footprints: a C nucleotide from each side of the junction was changed to G (Figure 4, B and C). Next, we identified the target site duplications from these two alleles. The results of Southern blot analysis (Figure 2) indicated the approximate transposon insertion sites for each allele; we then performed PCR using primers complementary to the *Ac* termini and the *p1* genomic sequences around the suspected integration sites to amplify the *Ac/p1* junction fragments in each mutant. For *p1-vv22*, the junction with the *Ac* 5′ end was PCR amplified using primer pair p1-7 + Ac-4. The PCR product was sequenced and found to contain the *Ac* 5′ end joined to *p1* genomic sequences located 4552 bp upstream of the transcription start site. The *p1* se-

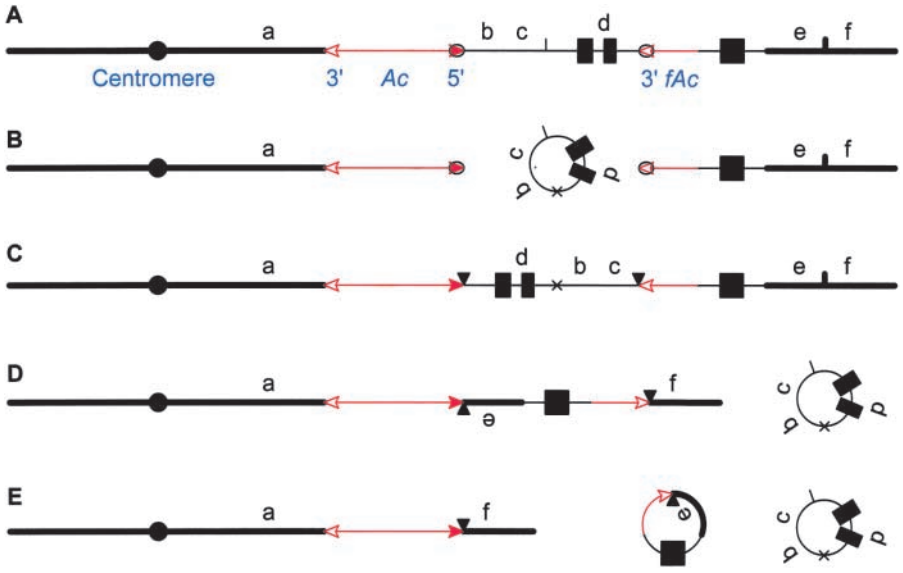

FIGURE 3.—Reversed-*Ac*-ends transposition model (see also animation at http://www.genetics.org/supplemental/). Solid boxes indicate *p1* gene exons 1, 2, and 3 (left to right). Red arrows indicate *Ac* or *fAc*; the open and solid arrowheads indicate the 3′ and 5′ ends, respectively, of *Ac*/*fAc*. Ovals indicate *Ac* transposase. Short vertical lines indicate transposon reinsertion sites, and solid triangles indicate TSD. (A) *Ac* transposase cleaves at the 5′ end of *Ac* and the 3′ end of *fAc*. (B) Following transposase cleavage at the junctions of *Ac*/*p1* and *fAc*/*p1*, the internal *p1* genomic sequences are joined to form a 13-kb circle. The "×" on the circle indicates the site where the joining occurred, marked by a transposon footprint. The *Ac* 5′ and *fAc* 3′ ends are competent for insertion anywhere in the genome. (C–E) The structures expected from insertion into two possible target sites. (C) The transposon ends insert into the 13-kb circle. The *Ac* 5′ end joins to the end adjacent to exon 1 and *fAc* 3′ end joins to the other end. The 13-kb sequence is rearranged (segment b–c and segment d exchanged positions). (D) The transposon ends insert distal to sequence e: the *Ac* 5′ end joins to the end adjacent to e, and the *fAc* 3′ end joins to the other end. Segment e is inverted, and the 13-kb circle is lost. The resulting chromosome contains an inversion of sequences from the *fAc* 3′ end to the insertion site distal to e. (E) The transposon ends insert distal to sequence e: the *fAc* 3′ end joins to the end adjacent to e, and the *Ac* 5′ end joins to the other end. Segment e is circularized and presumed lost; the 13-kb circle is also lost. The resulting chromosome contains an interstitial deletion from the *Ac* 5′ end to the insertion site distal to e.

quences are in an inverted orientation relative to their original genomic context (Figure 4B). The junction with the 3′ *fAc* end was amplified using primers p1-8 + Ac-2 and found to contain the 3′ end of *fAc* joined to *p1* sequences beginning immediately downstream of the site in the *p1* genomic DNA where the *Ac* 5′ end had inserted. Moreover, both junctions of the 5′ *Ac* and 3′ *fAc* termini are bounded by an 8-bp sequence (CAGAG TAT), which is found once at the original insertion site (Figure 4, A and B). This represents the 8-bp TSD characteristic of *Ac* insertions. For *p1-vv30*, we used nested primer pairs, p1-12 + Ac-4 and p1-11 + Ac-5, to amplify the new *p1-Ac* 5′ junction and p1-9 + Ac-3 and p1-10 + Ac-2 to amplify the new *p1-fAc* 3′ junction. Sequences of the PCR products show that the *Ac* 5′ end and *fAc* 3′ end are inserted into a site located 22 bp upstream of the *p1* transcription start site. Additionally, both *Ac*/*p1* junctions are bounded by the sequence CCGGCCGT, which represents an 8-bp TSD (Figure 4C). These results demonstrate that the *p1-vv22* and *p1-vv30* alleles were indeed generated by transposition reactions involving reversed *Ac* ends. The results are

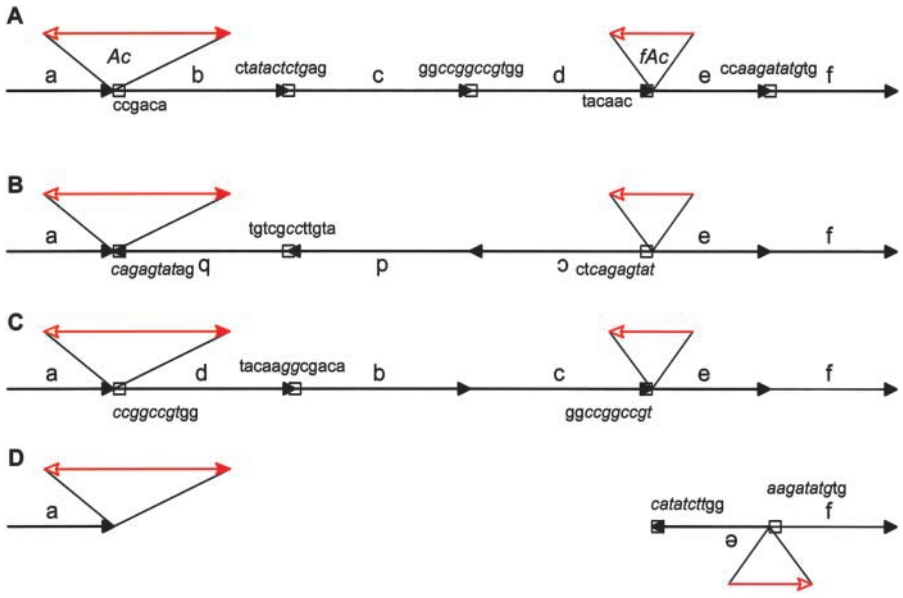

FIGURE 4.—Schematic structures of the *p1* region in *P1-rr11* and derivative alleles. (A) *P1-rr11*. Arrows in a–f indicate the various genomic segments of the *p1* gene. Boxes indicate the positions of the given sequences. (B) *p1-vv22*. Genomic segments b, c, and d have changed position and/or orientation. (C) *p1-vv30*. Genomic segments b, c, and d have changed position. (D) *p1-ww21*. Genomic segments b, c, and d were deleted; *fAc* and genomic segment e are inverted.

consistent with an intramolecular transposition model in which transposon excision produced a covalently closed circle composed of the 13-kb DNA between the reversed transposon ends, followed by insertion of the *Ac* 5′ and *fAc* 3′ ends into the circle. The resulting chromosome carries a rearrangement in which the 13-kb sequences between the *Ac* 5′ and *fAc* 3′ ends are circularly permuted. Depending upon the direction in which the transposon ends insert into the circle, the intervening DNA can be in the inverted or direct orientation, as for *p1-vv22* and *p1-vv30*, respectively. It is interesting to note that in both the *p1-vv22* and *p1-vv30* alleles, the 5′ end of *Ac* and the 3′ end of *fAc* are still competent for transposition. It is possible that in further reversed-ends transposition events, the transposon ends could reinsert into the *p1* intron 2 sequences in the correct orientation so as to restore *p1* function. Consistent with this idea, the *p1-vv22* and *p1-vv30* alleles are distinctive in exhibiting occasional red sectors that are not observed with the *p1-ww* alleles (Figure 1B).

Generation of inversions: The reversed *Ac* ends transposition model (Figure 3) predicts that insertion of the transposon ends into target sites in the flanking chromosomal regions would produce deletions and inversions. Southern blot analysis (Figure 2) suggested that the *p1-ww21* allele could be a small inversion caused by insertion of the transposon ends into a site a short distance downstream of the *p1* gene. To test this prediction, primer pairs p1-14 + Ac-4 and p1-15 + Ac-2 were used to amplify the new *Ac-p1* and *fAc-p1* junctions, respectively. Direct sequencing of the PCR products demonstrated that the insertion site is 496 bp downstream of *p1* exon 3 (Figure 4D); both insertion junctions are bounded by an 8-bp TSD (CATATCTT). Moreover, both the *fAc* and the 3′ flanking *p1* sequences (segment e in Figure 4D) are inverted in orientation with respect to the *Ac* 5′ end and sequences downstream of the insertion site (segment f in Figure 4D). These data, together with the Southern blot data indicating that *p1* fragment 8B is deleted (Figure 2, *Kpn*I-8B, and *Hin*dIII-8B), indicate that *p1-ww21* has a deletion and inversion as shown in Figure 4D. The rearrangement disrupted *p1* fragment 15, consistent with the *Hin*dIII-15 Southern blot results (Figure 2) of a 4-kb band (contains 281-bp *p1* fragment 15 and the downstream sequence homologous to fragment 15) and a faint 5.5-kb band (contains only 136 bp of fragment 15 sequence).

We isolated six additional *p1-ww* alleles (*p1-ww23, p1-ww24, p1-ww26, p1-ww31, p1-ww33*, and *p1-ww35*), which are deleted for the sequences between *Ac* and *fAc* (Figure 2, *Kpn*I-8B). Each of these alleles exhibits a new, different-sized band derived from the junction of 3′ *fAc* with the flanking DNA (Figure 2, *Kpn*I-15). However, all of these alleles retain the 6.5-kb *Hin*dIII fragment derived from *Hin*dIII sites in *fAc* and the *p1* 3′ flanking sequence (*Hin*dIII-15 blot in Figure 2). Additionally, PCR analysis using primer pair p1-1 + Ac-2 shows that the junction of the 3′ end of *Ac* and the *p1* 5′ flanking sequence is intact (not shown). The simplest explanation of these results is that these alleles carry inversions with endpoints outside of the *p1* locus to either side. As shown in Figure 3, inversions of this type would retain the original junctions of the *Ac* 3′ end and *fAc* with the outside flanking DNA, while the *Ac* 5′ end and the *fAc* 3′ end would be joined to new sequences. Similar structural data could also be obtained from reciprocal translocations; however, these alleles do not exhibit a semisterile phenotype as would be expected for translocation heterozygotes.

Generation of deletions: As shown in Figure 3, the reversed-ends transposition model predicts that flanking deletions would be generated as a consequence of insertion into a flanking target site. We identified three cases of deletions that extend to sites outside of the *Ac* or *fAc* insertion sites. In *p1-ww2052* and *p1-ww2053*, *p1* fragment 15 and *p1* fragment 8B are deleted, but the 8B-homologous sequence in the upstream paralagous gene *p2* is intact (Figure 2; Zhang *et al.* 2000). PCR analysis using primer pair p1-1 + Ac-2 shows that the junction of the 3′ end of *Ac* and the *p1* 5′ flanking sequence is intact (not shown). Therefore, these alleles most likely contain deletions caused by insertion of the 5′ *Ac* end at a site downstream of the *p1* fragment 15 homologous sequence. In *p1-ww25*, both *p1* fragment 8B and the *p2* sequence homologous to 8B are deleted (Figure 2), whereas sequences on the 3′ side of *p1* are intact, as evidenced by the 6.5-kb *Hin*dIII fragment detected by *p1* fragment 15. Therefore, this allele most likely contains a deletion caused by insertion of the 3′ *fAc* end into a site upstream of the *p2* gene. This result is consistent with our observation that *p1-ww25* lacks the silk-browning phenotype that is conferred by the presence of either *p1* or *p2* genes (Zhang *et al.* 2003).

DISCUSSION

We show here that a pair of *Ac* termini in reversed orientation and separated by 13 kb can undergo transposition reactions to generate chromosomal rearrangements including deletions, inversions, and a novel local rearrangement. The deletions extend from the maize *p1* locus to proximal or distal sites, and in one case removed a linked gene that is >20 kb distant. In addition, we isolated one inversion in which a 4879-bp fragment on the distal (downstream) side of *p1* was inverted, and six additional cases that are consistent with larger inversions on either side of the *p1* locus. Finally, we identified two cases in which the sequences between the reversed *Ac* termini were rearranged. All of these examples are consistent with a model in which *Ac* transposase utilizes two *Ac* termini in reversed orientation from different elements as transposition substrates. In this configuration, excision and reinsertion of the transposon termini directly generate the observed re-

arrangements. This model was confirmed by showing that the rearrangement breakpoints are delineated by the excision footprint and insertion TSD sequences that are the signatures of *Ac/Ds* transposition reactions. Thus, the rearrangements we characterized are produced directly from transposition reactions and are not the result of ordinary transposition followed by subsequent repair reactions (Athma and Peterson 1991; Xiao *et al.* 2000) or the result of ectopic recombination of TE sequences.

Previous research demonstrated that transposition of a single *Ac* element from various insertion sites in the maize *p1* gene can stimulate recombination between 5.2-kbp direct repeat sequences that flank the maize *p1* gene, resulting in a high frequency of *p1* deletions. These deletions were proposed to occur due to the generation of a double-strand break by *Ac* excision, followed by synthesis-dependent strand annealing-type repair involving the *p1* flanking repeat sequences (Athma and Peterson 1991; Xiao *et al.* 2000). However, none of the *p1* mutants derived from *P1-rr11* are deletions resulting from recombination between the flanking repeats; instead, all of the rearrangement endpoints characterized have precise junctions including TSD and footprint sequences that are characteristic of transposase-catalyzed break-and-join events. This suggests that transposition involving the *Ac* and *fAc* ends in maize does not generate free DNA ends that can participate in subsequent repair pathways. This contrasts with a recent report of large deletions induced by *Ds* transposition in Arabidopsis; these have relatively imprecise endpoints that appear to be generated by *Ds* excision followed by double-strand-break repair processes (Page *et al.* 2004).

The new rearrangement breakpoints of the alleles derived by reversed-*Ac* ends transposition are determined by the site into which the *Ac* termini insert. While *Ac/Ds* elements show a preference for transposition to nearby sites, a significant number of transpositions occur to sites a great distance away on the same chromosome or on another chromosome (Brutnell and Conrad 2003). Thus, the reversed-ends transposition mechanism described here may have the potential for generating very large-scale genome rearrangements. The maize *Ac/Ds* system has been shown to transpose in a large number of plants, including both monocot and dicot species; hence we expect that the reversed-ends transposition reaction described here could be similarly reproduced in a variety of transgenic plant systems. Interstitial deletions are potentially very useful for determining the functions of individual genes in multicopy gene clusters (Zhang *et al.* 2003). Although very large deletions would likely remove essential genes and thus be lethal, large inversions should be viable. Because recombination between a normal chromosome and an inversion-bearing chromosome in the region of the inversion generates genetically inviable gametes, large inversions are known as crossover suppressors. Chromosomes with multiple large inversions (balancer chromosomes) are used extensively in Drosophila to prevent the formation of recombinant gametes. Similarly, chromosomes containing large inversions could be generated in plants by reversed-ends transposition and used to eliminate crossover products in various genetic experiments.

Frequency and possible mechanism of reversed-ends transposition events: The cases we isolated were derived from an allele (*P1-rr11*) that contains a fractured *Ac* element (*fAc*) inserted into the *p1* gene intron 2 and an intact *Ac* element located ~9 kb upstream of the *p1* transcription start site. Neither the *Ac* nor the *fAc* elements in *P1-rr11* disrupt *p1* function; however, transposition reactions involving the 5′ end of the intact *Ac* and the 3′ end of the *fAc* will result in deletion or rearrangement of the *p1* gene promoter, exon 1, and exon 2 and thus generate colorless loss-of-function sectors on the otherwise red kernel pericarp. These sectors appear at a frequency of ~1 per kernel, depending on genetic background. The size of each sector reflects the time during development at which mutation occurs and ranges from very small (<1 mm) to large multikernel sectors. In contrast, simple transposition of the upstream intact *Ac* element is expected to occur more frequently (~10 sectors/kernel if similar to the transposition frequency of the *p1-vv* allele); however, excision of the upstream *Ac* element would not affect *p1* expression and thus would be undetectable in our screen. Further, the *fAc* element in *p1* intron 2 containing only the 3′ end of *Ac* is not competent to transpose, nor does it induce any instability in *p1* expression either alone or in the presence of an *Ac* element on another chromosome (not shown).

The 5′ end of the upstream intact *Ac* element could participate in transposition reactions with either the 3′ end of the same element or the 3′ end contributed by the downstream *fAc* element. It is not clear what determines which 3′ end is used in the transposition reaction. It has been proposed that assembly of *Ac/Ds* transposition complexes occurs during chromosomal DNA replication such that assembly is initiated with the first transposon end to be replicated and then completed using the next-replicated transposition-competent end (Xiao and Peterson 2002). According to this model, replication of *P1-rr11* in the distal to proximal direction (from 3′ to 5′ with respect to the *p1* gene) would replicate the 3′ end of *fAc* first, followed by the 5′ end of the intact upstream *Ac* element; these two ends would then interact and undergo reversed-ends transposition. However, replication in the proximal-to-distal direction (5′ to 3′ with respect to the *p1* gene) would result in assembly of a transposition complex using the 3′ and 5′ ends of the intact upstream *Ac* element, leading to standard transposition of the upstream *Ac* element. This model is consistent with recent

evidence that transposition is greatly enhanced by DNA replication (McElroy *et al.* 1997; Wirtz *et al.* 1997) and that competence for transposition is determined by differential binding of transposase to specific hemimethylated strands of the *Ac* termini (Ros and Kunze 2001). However, this model is not consistent with the observation that a pair of closely linked *Ds* insertions at the maize *Waxy* locus can undergo alternative transposition reactions resulting in chromosome breakage (Weil and Wessler 1993).

It is notable that 2 of the 12 cases studied here contained insertions of the *Ac* and *fAc* termini into the 13-kb sequence located between the transposon endpoints. This seems to be an unusually high frequency, even considering the well-known tendency of *Ac* to transpose to linked sites (Greenblatt 1984). Possibly, the excised circle formed upon reversed-ends transposition is topologically linked with the chromatid from which it was excised and thus may be held in close proximity to the transposon ends, making it a preferred target for reinsertion. Circular extrachromosomal *Ac* and *Ds* molecules have been described previously (Gorbunova and Levy 1997, 2000), although their origin and possible relation to the circular molecules discussed here is unclear.

Potential role of unconventional transposition in genome evolution: Numerous examples have shown that transposition can be accompanied by a variety of structural rearrangements. For example, McClintock described two cases in which transposition of *Ds* was associated with duplication of large segments of the short arm of maize chromosome 9 (McClintock 1951). Chromosome-breaking structures in maize typically contain multiple *Ac* and/or *Ds* termini; chromosome breakage has been proposed to occur through the transposition of a partially replicated macrotransposon (Ralston *et al.* 1989) or following fusion of sister chromatids in alternative transposition reactions (Weil and Wessler 1993). Support for the latter model has been obtained from analysis of transgenic tobacco showing that a pair of *Ac/Ds* 5′ and 3′ ends in direct orientation is capable of chromosome breakage (English *et al.* 1993, 1995) as a consequence of transposition reactions that act upon 5′ or 3′ *Ac* ends on different sister chromatids, leading to sister chromatid fusion. We previously presented evidence from twin sector analysis for a similar type of mechanism, termed sister chromatid transposition (previously termed nonlinear transposition; Zhang and Peterson 1999). In Drosophila, transposition reactions involving termini of *P* elements on different sister chromatids or homologous chromosomes can generate deletions and duplications (Gray 2000; Gray *et al.* 1996; Preston *et al.* 1996). Additionally, transposition involving termini of different *Tc1-mariner* elements in direct orientation generates deletions and inversions in Fusarium (Hua-Van *et al.* 2002). In contrast, the mechanism described here involves transposition of *Ac* ends in reversed orientation. To our knowledge, this configuration of transposon ends has not been directly tested for a eukaryotic transposon.

The rearrangements we describe here were generated through unconventional transposition reactions utilizing as substrates a pair of reversed *Ac* ends located 13 kb apart. Whenever two intact *Ac/Ds* elements are located on the same molecule in direct orientation, their terminal sequences adjacent to each other are in reversed orientation. This configuration can occur naturally when *Ac* transposes during DNA replication from a site that is already replicated to a closely linked but not-yet-replicated site. This mode of transposition generates genetically distinct daughter chromosomes, leading to the formation of twinned sectors, and is commonly observed for *Ac* transposition from the *p1-vv* allele (Greenblatt 1984). Similarly, *Ac/Ds* transpositions from the *bz1* locus on maize chromosome 9s can generate pairs of linked elements, and these have been shown to induce chromosome breakage (Dooner and Belachew 1991). However, it is not yet known whether the chromosome 9 breaks are caused by reversed-ends transposition or an alternative model involving transposition of a partially replicated macrotransposon (Ralston *et al.* 1989). In addition, transformation of plants with *Ac/Ds*-containing transgenes via particle-bombardment or Agrobacterium-mediated methods often results in multiple copies integrated in close proximity (Frame *et al.* 2002). It is possible that mutations derived from these types of structures may include rearrangements of the type described here.

The *Ac/Ds* elements are members of the *hAT* family of eukaryotic transposons, which are widespread in the animal and plant kingdoms (Kunze and Weil 2002). Due to the similarity in the basic biochemical features of the *hAT* transposition reaction, it seems likely that reversed termini of other *hAT* family members, and possibly other transposons, are able to transpose in maize and other organisms. This activity could, during the course of evolution, generate a variety of large-scale chromosomal rearrangements. Some of these, such as inversions and translocations, commonly result in partial sterility and may thereby represent an initial step in reproductive isolation and speciation (Lewis 1966). Thus, our results suggest that transposon-induced chromosomal rearrangements could play an important role in macroevolution and the origin of new species. Recent reports indicating that rearrangement breakpoints are often associated with repetitive sequences are consistent with this hypothesis (Evgen'ev *et al.* 2000; Coghlan and Wolfe 2002; Dunham *et al.* 2002).

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