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A Segmental Deletion Series Generated by Sister-Chromatid Transposition of *Ac* Transposable Elements in Maize

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ABSTRACT

Certain configurations of maize *Ac/Ds* transposon termini can undergo alternative transposition reactions leading to chromosome breakage and various types of stable chromosome rearrangements. Here, we show that a particular allele of the maize *p1* gene containing an intact *Ac* element and a nearby terminally deleted *Ac* element (*fAc*) can undergo sister-chromatid transposition (SCT) reactions that generate large flanking deletions. Among 35 deletions characterized, all begin at the *Ac* termini in the *p1* gene and extend to various flanking sites proximal to *p1*. The deletions range in size from the smallest of 12,567 bp to the largest of >4.6 cM; >80% of the deletions removed the *p2* gene, a paralog of *p1* located ~60 kb from *p1* in the *p1-vv* allele and its derivatives. Sequencing of representative cases shows that the deletions have precise junctions between the transposon termini and the flanking genomic sequences. These results show that SCT events can efficiently generate interstitial deletions that are useful for *in vivo* dissection of local genome regions and for the rapid correlation of genetic and physical maps. Finally, we discuss evidence suggesting that deletions induced by alternative transposition reactions can occur at other genomic loci, indicating that this mechanism may have had a significant impact on genome evolution.

DELETIONS have long been recognized as very efficient tools for genetic mapping. One of the best examples of the use of deletions for genetic fine-structure analysis is Benzer's classic work on the phage T4 *rII* gene. In this study, ~2400 *rII* mutants were first crossed with seven overlapping deletions that span the *rII* region. On the basis of their ability to generate functional recombinants, all the mutants were easily and unambiguously localized to one of the seven major deletion intervals. Further crosses of the mutants with smaller deletions in each of the major deletion intervals yielded more precise map data. By this approach, the relative order and position of the ~2400 mutants were determined using 25,000 crosses (Benzer 1961, 1962), whereas >2 million crosses would have been required to obtain the same results using a two- or three-factor method. Similarly, deletions have been successfully used for the physical mapping of part of the Drosophila X chromosome (Snyder *et al.* 1985) and for localization of the lettuce *dm3* mutation (Meyers *et al.* 1998).

In addition to genetic mapping, deletions are also useful for mutation screening in diploid organisms due to their pseudodominance phenotype. If a deletion heterozygote is used as starting material to perform mutagenesis, any nonlethal recessive mutation located within the deleted region can be detected in the M_0 generation; otherwise recessive mutations can be detected only in the following M_1 generation when they become homozygous (Klug and Cummings 1991).

The most widely used treatment to induce deletions is gamma irradiation (Anderson *et al.* 1996; Cecchini *et al.* 1998). However, high-energy irradiation can also induce other undesirable chromosome rearrangements and point mutations that can complicate the recovery and analysis of deletion mutants. In maize, the *r-X1* allele can induce terminal deletions, but the viability of large terminal deletions is poor (Birchler and Levin 1991; Lin *et al.* 1997). For target genes that are not near telomeres, in most cases it will not be possible to recover a viable deletion large enough to include the gene.

Recently, the cre/*lox* site-specific recombination system was used to generate deletions of up to 3–4 cM in mice (Ramirez-Solis *et al.* 1995; Li *et al.* 1996; Wagner *et al.* 1997; Zeh *et al.* 1998). The cre/*lox* system has also been applied to plant species such as tobacco and Arabidopsis to generate deletions, inversions, and reciprocal translocations (Dale and Ow 1990; Bayley *et al.* 1992; Russell *et al.* 1992; Odell *et al.* 1994; Medberry *et al.* 1995; Osborne *et al.* 1995). Deletions have been generated in plants using cre/*lox* and the *Ac/Ds* transposable element system as follows: Plants were transformed with a construct containing two *lox* sites—one *lox* site within a *Ds* element and a second *lox* site

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within the transgene, but outside *Ds*. In the presence of *Ac*-encoded transposase, the *Ds* element in the construct can transpose to a new site in the genome. Subsequent expression of cre recombinase can induce recombination between the *lox* sites in the original transgene locus and the transposed *Ds* element. If the transposed *Ds* element is on the same chromosome as the original transgene insertion, cre-induced recombination of the *lox* sites will generate either a deletion or an inversion, depending upon the relative orientation of the *lox* sites. If *Ds* transposed to another chromosome, cre-induced recombination will produce a reciprocal translocation. Using this system, researchers have produced a number of deletions in tobacco and Arabidopsis (Medberry *et al.* 1995; S. Zhang *et al.* 2003).

Previously we have shown that large deletions and inverted duplications could be generated in maize via transposition reactions involving *Ac/Ds* termini located on sister chromatids [sister-chromatid transposition (SCT), previously termed nonlinear transposition; Zhang and Peterson 1999]. The maize *p1-vv9D9A* allele carries an intact *Ac* element and a terminally deleted *Ac* element with their 5′ and 3′ termini in direct orientation. In the SCT model, *Ac* transposase can excise the 3′ *fAc* terminus and the 5′ *Ac* terminus on the two sister chromatids. The two chromatid ends at the site of *Ac/fAc* excision are ligated together as in a standard *Ac* transposition reaction, forming a covalent linkage between the sister chromatids (chromatid bridge). Reinsertion of the excised transposon ends into the chromatid bridge generates structurally altered sister chromatids containing a reciprocal deletion and inverted duplication (Zhang and Peterson 1999) (Figure 1; see also an animated version in supplemental material at http://www.genetics.org/supplemental/). In this article, we describe the isolation and molecular characterization of 35 interstitial deletions derived from the *p1-vv9D9A* allele. As predicted by the SCT model, all the deletions start at the *Ac/fAc* insertion site in *p1-vv9D9A* and end at various sites in the region proximal to the *p1* locus. These results provide further support for the SCT mechanism and demonstrate the utility of SCT for the production of deletions in plants.

MATERIALS AND METHODS

Mutation screening: The maize *p1* gene controls red phlobaphene pigmentation of husks and floral organs, including kernel pericarp and cob glumes. The *p1-vv9D9A* allele confers variegated pericarp and cob (Zhang and Peterson 1999), and the *P1-wr* allele confers colorless pericarp and red cob (Anderson 1924). The *r-m3::Ds* allele contains a *Ds* element inserted in the *r1* gene required for kernel aleurone pigmentation; *Ac*-induced excision of *Ds* from *r-m3::Ds* results in purple aleurone sectors (Kermicle 1980). SCT reactions involving the *p1-vv9D9A* allele are predicted to result in deletions extending from the *Ac/fAc* insertions in *p1* intron 2 toward the 5′-end of the *p1* gene. Deletions that extend into and beyond exons 1 and 2 would remove the Myb-homologous DNA-binding domain and thus should abolish *p1* function, leading to a p1-ww phenotype (colorless pericarp and cob). Therefore, we screened ears from plants of genotype *p1-vv9D9A/P1-wr* pollinated with *P1-wr, r-m3::Ds* for multiple-kernel sectors of colorless pericarp or whole colorless-pericarp ears. From a total of 4000 ears produced on plants grown in two generations, we obtained 45 ears with large multiple-kernel colorless pericarp sectors, 54 ears with completely colorless pericarp, and 1 ear with a large twinned colorless pericarp sector, described in Zhang and Peterson (1999). From the colorless pericarp sectors, we selected purple-spotted kernels for progeny analysis as these were predicted to contain an *Ac* element linked with the desired deletion alleles. Plants grown from these kernels were self-pollinated to homozygose the new *p1-ww* alleles. In the following generation, plants were screened for the presence of colorless tassel glume margins to distinguish homozygous *p1-ww* plants from sibling plants heterozygous or homozygous for the *P1-wr* allele, as described previously (Athma and Peterson 1991). In addition, putative mutant plants were also screened for the occurrence of browning at the cut ends of silks (Levings and Stuber 1971), an indicator of the presence of maysin, a C-glycosyl flavone whose synthesis is coregulated by the *p1* and *p2* genes (Byrne *et al.* 1996; P. Zhang *et al.* 2003; Szalma *et al.* 2005). New mutant alleles with colorless pericarp and cob were designated *p1-ww*, followed by a numerical indicator of culture number, according to standard nomenclature. The alleles *p1-ww1* and *p1-ww2* described here were formerly named *p1-ww-def1* (Zhang and Peterson 1999) and *p1-del2* (P. Zhang *et al.* 2003).

Genomic DNA extractions and Southern blot hybridization: Total genomic DNA was prepared using a modified CTAB extraction protocol (Saghai-Maroof *et al.* 1984). Agarose gel electrophoresis and Southern hybridizations were performed as described (Sambrook *et al.* 1989), except that 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. The RFLP probes csu814, npi286, csu392, and asg69 were provided by T. Muskett and M. McMullen, University of Missouri, Columbia, Missouri. Hybridization signals were quantified using ImageQuant 5.0.

PCR amplifications: PCR amplifications were performed using the following oligonucleotide primers: p1-1, ATCCAT CGCCCAACCCCAACC; p1-2, TGAACACTAAATACTCAATC GTGGCAT; p1-3, ACGCGCGACCAGCTGCTAACCGTG; p1-4, GAATTCCGCCCGAAGGTAGTTGATCC; p1-5, CTGGCGAG CTATCAAACAGGACA; Ac6, ATTTTACCGACCGTTACCGA CC; Ac7, ATCTTCCACTCCTCGGCTTTAG; and p1-8, GACC GTGACCTGTCCGCTC. Reactions were heated at 94° for 3 min, cycled 35 times at 94° for 20 sec, 60° for 30 sec, and 72° for 1 min per 1-kb length of expected PCR product, and finally extended at 72° for 8 min.

Pulsed-field gel electrophoresis: Characterization of high-molecular-weight maize genomic DNA was done following the protocols described by (Kaszas and Birchler 1996, 1998). Pulsed-field gel electrophoresis (PFGE) was conducted on a CHEF-DRII apparatus (Bio-Rad, Hercules, CA), and membranes were hybridized as described above.

RESULTS AND DISCUSSION

Identification of SCT-generated deletion mutants: Upon sister-chromatid transposition involving the *Ac* and *fAc* elements in *p1-vv9D9A*, the excised *Ac/fAc* ends could, theoretically, reinsert anywhere in the maize

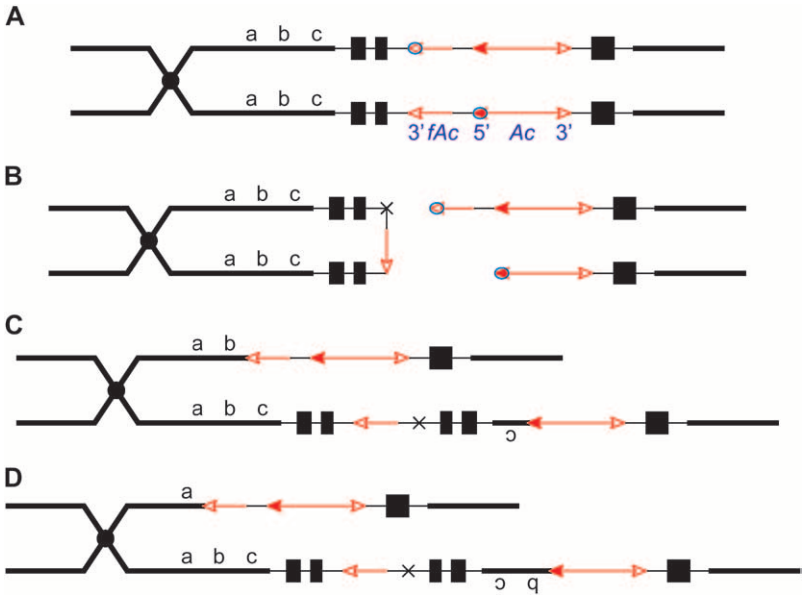

FIGURE 1.—Model for formation of deletions by sister-chromatid transposition. (For animated version, see supplemental material at http://www.genetics.org/supplemental/.) The diagram pertains to the structure of the *p1-vv9D9A* allele, which is the progenitor of the *p1-ww* deletion alleles described in the text. The two lines indicate sister chromatids joined at the centromere, which is indicated by a solid circle. The solid black boxes indicate the three exons of the *p1* gene; the 5′-end of the *p1* gene is closer to the centromere (ZHANG and PETERSON 1999). The red arrows indicate the *Ac* or *fAc* elements inserted into the second intron of the *p1* gene, and the open and solid arrowheads indicate the 3′- and 5′-ends, respectively, of *Ac/fAc*. The short black line between *Ac* and *fAc* indicates a 112-bp rearranged *p1* sequence (rP) present in the *p1-vv9D9A* allele (not to scale). (A) Following DNA replication, identical sister chromatids are joined at the centromere. *Ac* transposase (small circles) binds to the 5′ terminus of *Ac* in one chromatid and to the 3′ terminus of *fAc* in the sister chromatid. (B) Cuts are made at the *Ac* and *fAc* termini to excise the transposon ends. The two nontransposon ends join together at the site marked by the black **X** to form a chromatid bridge. (C) Reinsertion of the excised transposon ends into the chromatid bridge between b and c generates two reciprocal chromatids; one carries a deletion of c and the other carries an inverted duplication of c. (D) Same as for C, except that reinsertion between a and b generates one chromatid with a deletion of b c and one with an inverted duplication of b c. For simplicity, the model depicts fully replicated sister chromatids at the time of transposition. In reality, transposition may occur when the chromosomes are partially replicated.

genome. Insertions into sites distal to *p1* on chromosome 1s would be predicted to generate acentric molecules and large, nontransmissable, terminal deficiencies. However, insertions into sites proximal to *p1* would restore centromere linkage and generate sister chromatids containing reciprocal deletion and inverted duplication products as previously shown (ZHANG and PETERSON 1999) (Figure 1). If the *Ac/fAc* reinsertion site is in or upstream of exon 2 of *p1*, the *p1* gene function would be destroyed due to loss of exon 2, which encodes a part of the R2R3-Myb DNA-binding domain (GROTEWOLD *et al.* 1991). The resulting deletion mutants are expected to have a p1-ww phenotype (colorless pericarp and cob), which in large multiple-kernel sectors or whole ears is easily distinguishable from the variegated pericarp and cob phenotype of the *p1-vv9D9A* progenitor allele. However, not all *p1*-ww alleles derived from *p1-vv9D9A* are expected to contain SCT-generated deletions; at least three other types of structural changes in the *p1-vv9D9A* allele could also destroy *p1* function. First, the inverted duplication alleles (*p1-ww-id*) generated via SCT as the reciprocal product of deletions also have disrupted the *p1* gene and thus have a p1-ww phenotype (ZHANG and PETERSON 1999). Second, *p1-ww* alleles can arise from *Ac* transposition-induced recombination between two 5.2-kb direct repeat sequences that flank the *p1* gene, leading to the loss of the entire *p1* coding region (ATHMA and PETERSON 1991; XIAO *et al.* 2000). Third, *p1-ww* alleles might arise due to intragenic transposition of *Ac*, if the insertion and associated 8-bp target site duplication (TSD) occurred at an essential sequence of the *p1* gene (ATHMA *et al.* 1992; MORENO *et al.* 1992).

From 4000 ears produced by plants of genotype *p1-vv9D9A/P1-wr*, we selected 100 ears with completely colorless pericarp or with large multiple-kernel colorless pericarp sectors (see MATERIALS AND METHODS). The SCT model (Figure 1) predicts that SCT-induced deletions should lose sequences upstream of the *fAc* insertion site in *p1-vv9D9A*, while retaining the *p1* gene sequence downstream of the *Ac* sequence. In contrast, null alleles generated by recombination of the flanking repeats would lack the entire *p1* gene and *Ac* element (ATHMA and PETERSON 1991), while alleles carrying inverted duplications or new insertions of *Ac* would retain both upstream and downstream *p1* sequences. To distinguish SCT-generated deletions from these other classes of mutations, a multiplex PCR assay was performed to test for the presence of sequences upstream and downstream of the *fAc/Ac* insertions in the *p1* gene (Figure 2). Primers p1-1 + p1-2 were used to detect losses of the *p1* 5′ region, while primers Ac-6 + p1-8 were used to detect retention of the junction of *Ac* and the *p1* 3′ sequence. Moreover, the Ac-6 + p1-8 primer pair can serve as positive internal controls for this multiplex PCR assay. Among 100 *p1-ww* mutants screened in this assay, we identified 35 cases that exhibit a loss of the 5′ *p1* gene sequences, but retain the 3′ *p1* sequence as expected for SCT-generated deletions (Figure 2B).

Identification of deletions extending to the *p2* gene and beyond: The *p1* gene is linked with a second, highly similar gene termed *p2*; the *p1* and *p2* genes were

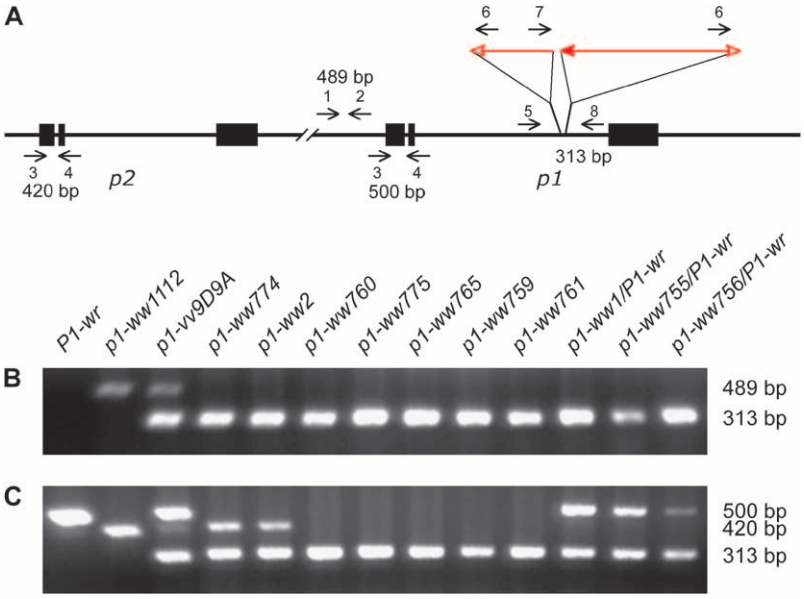

FIGURE 2.—Detection of deletions by PCR analysis. (A) Structure of the *p1-vv9D9A* haplotype, including *p1* (right) and its paralog *p2* (left). Symbols have the same meaning as in Figure 1. Short horizontal arrows indicate the orientations and approximate positions of the primers used in PCR analysis. (B) Screening for deletions of sequences 5′ of the *p1* gene using primer pair p1-1 + p1-2, which gives a 489-bp product in *p1-vv9D9A*. The primer pair Ac-6 + p1-8 detects a 313-bp band from the junction of the 3′-end of *Ac* with the 3′ sequence of *p1* intron 2. PCR was performed using genomic DNA from plants of the genotypes indicated above each lane. The lane marked *P1-wr* contains DNA from the W22 inbred. The *P1-wr* allele has been previously shown to contain a tandem array of *p1* genes (CHOPRA *et al.* 1998), whereas no *p2* gene was detected in 16 diverse maize inbred lines containing *P1-wr* (SZALMA *et al.* 2005). The negative result in the *P1-wr* lane would suggest that *P1-wr* alleles also lack (or are polymorphic for) the sequence upstream of *p1* in *p1-vv*. The *p1-ww1112* allele contains a deletion of *p1* (ATHMA and PETERSON 1991) and retains the *p2* gene (ZHANG *et al.* 2000). (C) Screening for deletions of the 5′-end of the *p2* gene using primer pair p1-3 + p1-4, which gives a 420-bp product from the *p2* gene and a 500-bp product from the *p1* gene. As in B, primer pair Ac-6 + p1-8 detects a 313-bp band derived from the junction of the 3′-end of *Ac* with the 3′ sequence of *p1* intron 2.

proposed to have been generated by a segmental duplication followed by retroelement insertions to separate the two paralogs (ZHANG *et al.* 2000). If the *p2* gene is located 5′ of *p1*, then some of the SCT-generated deletions would be expected to have deletions that include *p2*. Consistent with this hypothesis, a *p1-ww* allele derived from *p1-vv9D9A* (*p1-ww2*) was previously characterized and found to have a deletion extending from *p1* to the *p2* gene (P. ZHANG *et al.* 2003). To determine the frequency at which SCT deletions remove the *p2* gene, we used a second PCR assay with primers p1-3 + p1-4. This primer pair amplifies the 5′ region of both *p1* and *p2*; due to sequence polymorphisms, the products derived from *p2* and the *p1* alleles used in this cross differ in size. (Primers Ac-6 + p1-8 again serve the same role as in the first PCR assay.) Among 35 *p1-ww* deletion alleles tested, 6 alleles retain the 420-bp *p2* fragment whereas the other 29 *p1-ww* alleles lack this product (Figure 2C), suggesting that *p2* was deleted in these 29 cases. Among the 29 alleles with deletions of *p2*, 7 alleles are homozygous lethal. These 7 cases were maintained as heterozygotes with the *P1-wr* allele, which produces a 500-bp product in the PCR assay (Figure 2C).

The region in the vicinity of *p1* has been identified as a major QTL for the control of levels of silk maysin, a C-glycosyl flavone that deters feeding by corn earworm (BYRNE *et al.* 1996; LEE *et al.* 1998; MCMULLEN *et al.* 1998). Maysin accumulation is correlated with a phenotype termed silk browning, in which the cut ends of silks turn brown due to the oxidation of flavones (BYRNE *et al.* 1996; LEE *et al.* 1998; MCMULLEN *et al.* 1998; GUO *et al.* 2001; RECTOR *et al.* 2003). Both *p1* and *p2* genes are expressed in maize silk (ZHANG *et al.* 2000), and both encode highly similar R2R3-Myb proteins with a similar potential to activate flavonoid biosynthesis in transgenic cell lines (P. ZHANG *et al.* 2003). Previous studies have shown that a stock that contains both *p1* and *p2* genes has high maysin levels and strong silk browning. In contrast, a previously characterized deletion allele (*p1-ww774*), which has a deletion of *p1* but retains *p2*, conditions light-browning silks and low, but significant, maysin levels (P. ZHANG *et al.* 2003). To further test the role of the *p1* and *p2* genes in the control of silk maysin and silk browning, we examined the silk browning phenotype of 28 deletion lines that are homozygous viable (the remaining 7 deletions were not informative because they were maintained as heterozygotes with the *P1-wr* allele that specifies silk browning). Among the 22 homozygous-viable deletion lines that lack the 5′-ends of both the *p1* and *p2* genes, all had nonbrowning silks, whereas among the 6 deletion lines that lack the 5′-end of *p1* but retain the 5′-end of *p2*, 5 exhibited light-browning silks, and 1 line (*p1-ww2*) exhibited nonbrowning silks. Interestingly, this latter line contains a deletion into the 3′-end of the *p2* gene (see below). Although our data do not rule out the possibility of an additional factor involved in maysin biosynthesis located between *p1* and *p2*, the simplest interpretation of our results is that the *p2* gene is sufficient to confer weak maysin levels, while *p1* and *p2* together produce higher maysin levels and stronger silk browning. These results further support the hypothesis that the *p1* and *p2* genes are essential coregulators of maysin biosynthesis (BYRNE *et al.* 1996; P. ZHANG *et al.* 2003; SZALMA *et al.* 2005).

Sequences of deletion endpoints contain precise junctions with *Ac/fAc* termini: The SCT model predicts

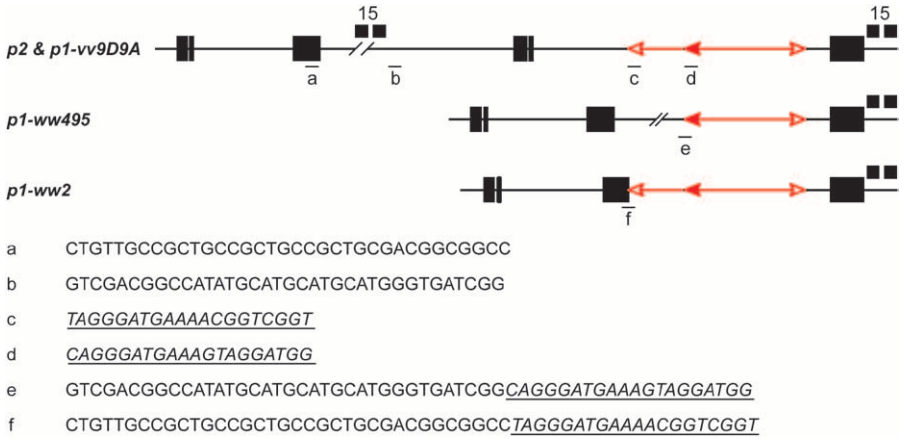

FIGURE 3.—Nucleotide sequences at endpoints of *p1-ww495* and *p1-ww2* deletion alleles. The top three lines show the structures of the indicated alleles and the locations of the sequences given below. Sequences a–d are from the progenitor allele *p1-vv9D9A*. Sequences e and f are from the derivative alleles *p1-ww495* and *p1-ww2*, respectively. Sequences in italics and underlined represent *Ac* or *fAc* sequences. Note that the deletion endpoint in *p1-ww495* is joined to the *Ac* 5′-end, while the deletion endpoint of *p1-ww2* is joined to the 3′ *fAc* end. Small black boxes indicate the locations of sequences that hybridize with *p1* genomic fragment 15. Other symbols have the same meaning as in Figure 1.

that deletion endpoints are determined by transposase-mediated insertion of *Ac* or *fAc* termini into flanking genomic sequences. If the SCT reaction is mechanistically similar to standard transposition, then the deletion endpoints should contain precise junctions of the *Ac* or *fAc* termini and the flanking genomic sequences with no loss of sequences at either the transposon termini or the genomic sequence. An 8-bp TSD is predicted to occur at the insertion site, with one copy present at the deletion endpoint and the second copy present in the inverted duplication structure formed as a reciprocal product of the SCT reaction (Figure 1). We previously demonstrated that an 8-bp TSD was present in both the deletion and the inverted duplication alleles generated from a single SCT event, indicating no loss of sequences at the genomic insertion site (ZHANG and PETERSON 1999). To further investigate the structures of deletion endpoints, we sequenced the junctions of the *Ac/fAc* termini with the genomic DNA in two additional cases. The first case (*p1-ww495*) was identified in the course of DNA gel-blot hybridizations with *p1* locus probes that were performed on a subset of deletions to check the results of the PCR assays presented in Figure 2. These results (not shown) suggested that *p1-ww495* had an endpoint upstream of *p1* within a region that was previously cloned and sequenced (AF209212). PCR using primers from the *Ac* and the *p1* genomic sequences flanking the estimated insertion site were used to amplify the *Ac/p1* junction. Sequencing of the PCR product indicated that the deletion junction occurred exactly at the 5′-end of *Ac* at a genomic site 12,567 bp upstream of the 5′-end of *Ac* (Figure 3). The second case (*p1-ww2*) was previously shown by DNA gel-blot analysis to have an endpoint in the 3′ region of the *p2* gene (P. ZHANG *et al.* 2003). This result is consistent with PCR analysis showing that *p1-ww2* lost the 5′ region of *p1* but retained the 5′ portion of *p2* (Figure 2). Further PCR and sequencing analysis indicated that the endpoint of *p1-ww2* is in exon 3 of the *p2* gene at a site 63 bp upstream of the *p2* translation stop codon (Figure 3; 9588 in AF210616) (P. ZHANG *et al.* 2003). In this case, the *p1-ww2* deletion endpoint occurs exactly at the *fAc* 3′-end. These results demonstrate that, at least in these three cases, the deletion endpoints occurred precisely at the *Ac* or the *fAc* terminus as predicted by the SCT model. It is possible that imprecise junctions exist among the 32 other deletions derived from *p1-vv9D9A*; however, the three junctions sequenced to date support the hypothesis that the SCT-induced deletion endpoints occur precisely at the site of insertion of the *Ac/fAc* termini.

In both maize and Arabidopsis, transposition of simple *Ac/Ds* elements can generate sequence changes (commonly termed footprints), including small deletions at the site of transposon excision (RINEHART *et al.* 1997). Evidence indicates that these sequence changes are the result of cellular functions acting to repair the site of transposon excision (YU *et al.* 2004). Deletions associated with excision of a simple transposon are usually relatively small (*i.e.*, <50 bp), although a deletion >700 bp associated with excision of a single *Ac* element has been reported (DOONER *et al.* 1988). Some large deletions have been found in maize following excision of compound elements composed of *Ac/Ds* termini flanking genomic sequences (RALSTON *et al.* 1989; DOWE *et al.* 1990). More recently, PAGE *et al.* (2004) identified several large deletions (≥100 kb) in Arabidopsis that were apparently generated during *Ac*-induced excision of a simple *Ds* element. The authors suggest that these large deletions were formed through a two-step process in which normal transposition of *Ds* is followed immediately by intrachromosomal excision of a hybrid *Ds* element. However, the deletion endpoints reported by PAGE *et al.* (2004) do not end precisely at the *Ds* termini, suggesting that the formation of these deletions probably involved other cellular functions in addition to the *Ac* transposase. Transposition of simple *Ac/Ds* elements is not known to induce large deletions in maize, and their occurrence in Arabidopsis may reflect a loss of normal transposition controls in the nonnative host.

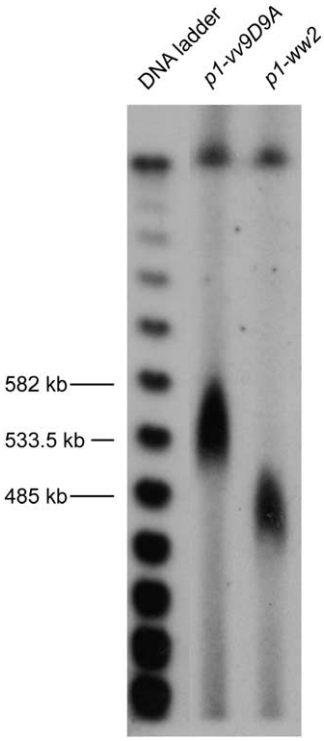

FIGURE 4.—Determination of the physical distance between *p1* and *p2* by CHEF gel analysis. Cells from plants of the indicated genotypes were protoplasted, embedded in agarose, and digested with *Not*I endonuclease. DNAs were separated by CHEF gel electrophoresis, transferred to membrane, and hybridized with genomic probe fragment 15 from the *p1* gene (Figure 3). Left lane contains λDNA concatemers as size standards.

Use of deletions to determine the physical distance between *p1* and *p2*: The fact that the *p2* gene is lost in 29 of 35 SCT-generated deletion alleles suggests that *p2* is tightly linked with *p1*. We used CHEF gel analysis to determine the physical distance between *p1* and *p2* by comparing the progenitor allele *p1-vv9D9A* with the deletion allele *p1-ww2* in which the deletion endpoint lies within the *p2* gene exon 3. Agarose blocks containing protoplasted cells from plants homozygous for *p1-vv9D9A* or *p1-ww2* were digested with *Not*I and subjected to CHEF gel electrophoresis. The DNA fragments were transferred to membranes and hybridized with *p1* fragment 15, which detects a sequence located both 5′ and 3′ of the *p1* gene (Figure 3). Because the SCT-induced deletions retain the *p1* 3′ sequence, fragment 15 can be used to detect the *Not*I fragments containing this sequence in both alleles. The size difference of the signals from the two alleles is ~70 kb (Figure 4). After accounting for the *p1* 5′ sequences and the *p2* 3′ sequences that are deleted in *p1-del2*, we estimate that the intergenic distance between the *p1* and *p2* genes is ~60 kb.

We previously reported that the *p1* gene is oriented with its 5′-end toward the centromere (ZHANG and PETERSON 1999). The *p1-ww2* allele has a deletion of the 5′ portion of the *p1* gene, the 3′ portion of the *p2* gene, and the intervening sequences. Assuming that no other rearrangements occurred during the formation of *p1-ww2*, we can infer that the *p2* gene has the same orientation as that of the *p1* gene and that it is located between *p1* and the centromere in the following arrangement: 3′-*p1*-5′, 3′-*p2*-5′, centromere. This conclusion is consistent with previous results showing that the *p1* and *p2* genes are derived from a segmental duplication, followed by retroelement insertions to separate the *p1* and *p2* genes (ZHANG *et al.* 2000).

Interval mapping of the SCT-generated deletions: To determine the relative sizes of the other deletions, six *p1*-linked probes (ndp1, ndp2, csu814, npi286, csu392, asg69) were used for genomic DNA gel-blot analysis of 10 representative deletions (including *p1-ww2*). We previously described genomic fragments ndp1 (formerly p1.5B22) and ndp2 (formerly pJZPX): ndp1 was isolated from the endpoint of inverted duplication allele *p1-ww12:27-3*, which was derived from *p1-vv9D9A*. ndp2 was isolated from a second inverted duplication allele, *p1-ww-id1*, also derived from *p1-vv9D9A*. ndp1 and ndp2 were mapped at 3.5 and 4.6 cM proximal to *p1*, respectively (ZHANG and PETERSON 1999). For Southern analysis, genomic DNA was digested with *Hin*dIII and hybridized with the ndp1 and ndp2 probes. Several alleles are homozygous inviable (see below), and these were tested as heterozygotes with a *p1-ww* allele from inbred line 4Co63. For ndp1, three alleles (*p1-ww1*, *p1-ww755*, and *p1-ww756*) contain a band of the same size as that in the *p1-ww* [4Co63] parent, but they lack the band corresponding to the DNA fragment from the chromosome carrying the *p1-vv9D9A* allele. The same three alleles also lack a band hybridizing with ndp2. We conclude that these alleles are deleted for the loci represented by the ndp1 and ndp2 probes (Figure 5A).

Probe csu814 was mapped to the same position as that of *p1*, and npi286, csu392, and asg69 were mapped 3.6, 4.7, and 5.6cM proximal to *p1*, respectively (http://www.maizegdb.org/cgi-bin/displaymaprecord.cgi?id=143431). Genomic DNA was digested with *Hin*dIII or *Sac*I, and the four RFLP markers were used as probes for Southern analysis. Probe csu814 produced a complex Southern pattern (not shown), but there was no evidence that this sequence was deleted in any of the alleles (not shown). This suggests that csu814 is probably distal to *p1*. Probe csu392 hybridized with two genomic *Hin*dIII restriction fragments, which are nonpolymorphic between the deletion stocks tested here and the 4Co63 inbred line (Figure 5A). The signal intensities of the two bands were measured using ImageQuant 5.0 (see supplemental Figure S1 at http://www.genetics.org/supplemental/). In Figure 5A, in lanes 1–8 (parental *p1-vv9D9A* and derivative alleles) and in lane 12 (inbred 4Co63), the signal for the upper band is slightly less than, or approximately equal to, that of the lower band, whereas in lanes 9–11 (deletion alleles *p1-ww1*, *p1-ww755*, and

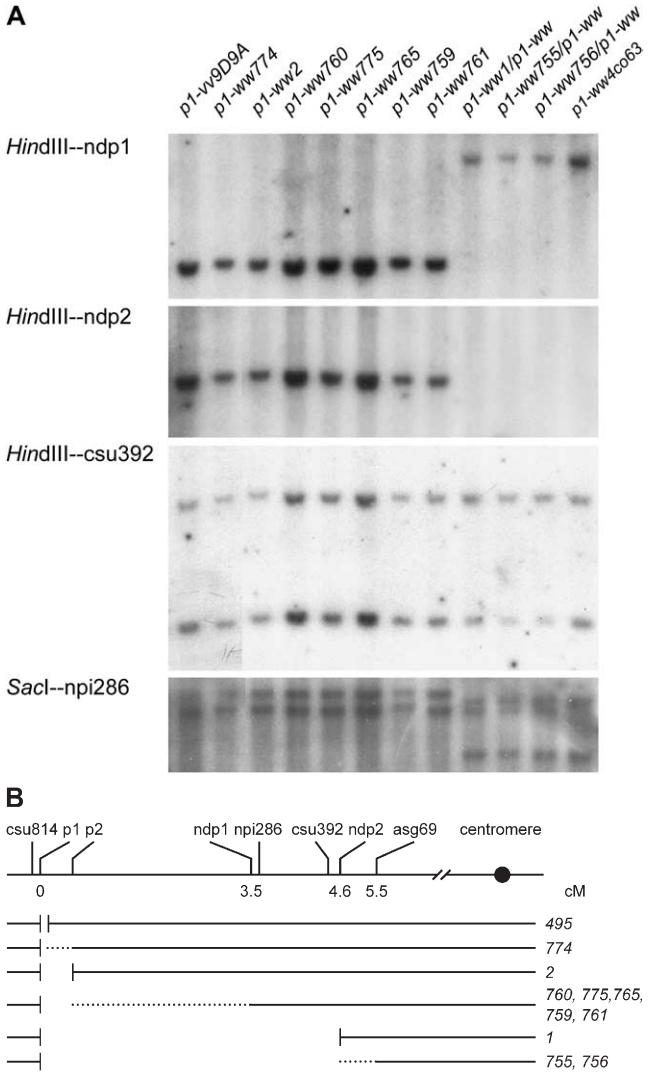

FIGURE 5.—Genomic DNA gel-blot analysis of the SCT-induced deletion alleles using probes linked to the *p1* gene. (A) Genomic DNA of the genotypes indicated above each lane was digested with *Hin*dIII or *Sac*I and hybridized with the indicated probes. See text for details. (B) Summary of endpoint mapping data of the SCT-generated *p1-ww* deletions. Schematic at top shows the positions of probe fragments (the solid boxes) and genetic distances in centimorgans, where known. Lines below show the extent of deletion found in the alleles indicated by the numbers to the right. Short vertical lines indicate deletion endpoints defined by cloned sequences. Dotted lines indicate the intervals into which those deletion(s) map. The relative order of ndp1 and npi286 cannot be determined on the basis of hybridization data reported here; the positions shown are based on prior recombination data showing a genetic distance from *p1* of 3.5 and 3.6 cM for Ndp1 and npi286, respectively (http://www.maizegdb.org/cgi-bin/displaymaprecord.cgi?id=143431). See text for details.

p1-ww756, each heterozygous with *p1-ww* [4Co63]) the signal of the upper band is approximately twofold greater than the signal from the lower band. These results suggest that the genomic *Hin*dIII fragment corresponding to the lower band is missing in these three alleles; the band of lower signal intensity in these lanes represents the corresponding *Hin*dIII fragment from the 4Co63 genotype. On the basis of this altered signal intensity, we infer that csu392 is probably deleted from the chromosomes carrying the deletion alleles *p1-ww1*, *p1-ww755*, and *p1-ww756*. According to the map data, csu392 is 0.1 cM proximal to ndp2; the fact that *p1-ww1* appears to be deleted for csu392 suggests that csu392 is actually distal to ndp2, because ndp2 was derived from sequences adjacent to the endpoint of *p1-ww1* (ZHANG and PETERSON 1999). Probe npi286 hybridizes with multiple bands; one of these bands is specifically missing in *p1-ww1*, *p1-ww755*, and *p1-ww756* (Figure 5A). For asg69, no deletion was detected in any of the alleles (data not shown here).

A summary of the mapping data based on these Southern hybridizations and other information is presented in Figure 5B. Probe csu814 was not deleted in any alleles and hence is probably distal to *p1*. The *p1-ww495* allele has the small (12,567 bp) deletion (described above), which ends just upstream of *p1*. The endpoint of *p1-ww774* is placed between *p1* and *p2*, and the *p1-ww2* deletion ends within the *p2* gene. Five deletion alleles (*p1-ww759*, *p1-ww760*, *p1-ww761*, *p1-ww765*, and *p1-ww775*) have endpoints in the interval between *p2* and ndp1. The *p1-ww1* allele has its endpoint adjacent to ndp2, and *p1-ww755* and *p1-ww756* have their endpoints between ndp2 and asg69. Finally, probes npi286 and csu392 are located in the interval between ndp1 and ndp2.

It is important to note that the genetic distances in the map presented in Figure 5B are based on previous genetic recombination data and are likely only approximate. However, the relative order of the markers indicated by the deletion mapping presented here should be robust, assuming that the deletions are simple and unidirectional.

Location of the *p1*-linked genes *zygotic lethal 1* and *defective kernel 1*: It was interesting to determine whether any of the deletions disrupted known genes in the vicinity of *p1*. The *zygotic lethal 1* (*zl1*) mutation was mapped 1.5 cM proximal to *p1* (EMERSON 1939). The *zl1* mutation does not affect viability of the male or female gametophyte, but it is homozygous lethal in the zygote. (The original *zl1* mutant stock has apparently been lost, and no other zygotic lethal mutations have been described to date in maize.) Interestingly, among the 35 *p1-ww* alleles studied here, 7 confer a zygotic lethal phenotype. One of these is the previously characterized *p1-ww1* allele. The *p1-ww1* allele transmits normally through both the pollen and the ovule; however, no homozygous *p1-ww1* plants were recovered from >120 progeny plants derived from the self-pollination of a *p1-ww1/P1-wr* plant. There is a negligible probability (1.01×10^{-15}) that homozygous *p1-ww1* plants were not recovered by chance from a planting of this size. Additionally, self-pollinated *p1-ww1/P1-wr* ears show some empty spaces and irregular kernel rows, which

are typical signs of 25% semisterility. For the remaining 6 *p1-ww* alleles, no *p1-ww* homozygous plants were identified among 20 or more progeny plants derived from self-pollination of plants carrying each *p1-ww* mutation heterozygous with *P1-wr*. The probability that *p1-ww* homozygotes were not detected by chance among 20 progeny of each self-pollinated *p1-ww/P1-wr* heterozygote is 0.3%. We conclude that the homozygous lethality of these 7 *p1-ww* alleles is probably due to loss of the *zl1* locus. Because we obtained 22 *p1-ww* alleles that removed *p2* and yet are viable as homozygotes, the *zl1* locus is placed on the centromeric side of *p2*. The smallest characterized deletion that has a zygotic lethal phenotype is *p1-ww1*, whose endpoint is at the site of probe ndp2. Thus, the *zl1* locus must lie in the interval between *p2* and ndp2, with the gene order of *p1*, *p2*, *zl1*, centromere.

A second gene known to be in the vicinity of *p1* is *defective kernel 1* (*dek1*), which was tentatively mapped at 0.8 cM proximal to *p1* (Dooner 1980). The *dek1* gene encodes a 2159-aa protein belonging to the calpain superfamily and is essential for kernel aleurone development (Becraft and Asuncion-Crabb 2000; Becraft *et al.* 2002; Lid *et al.* 2002; Wang *et al.* 2003). Some of the *p1-ww* deletions described here extend >4.6 cM proximal to *p1*; if *dek1* were 0.8 cM proximal to *p1*, then it should be deleted in some of these cases. However, no *dek1* kernels were obtained by self-pollination of any of the *p1-ww* alleles obtained in this study. To test whether the zygotic lethal phenotype of the seven largest deletions is the null phenotype of *dek1*, we crossed three large deletions, which conferred the zygotic lethal phenotype (*p1-ww1*, *p1-ww755*, and *p1-ww756*) to *dek1/Dek1* heterozygous plants; again, no *dek1* kernels were found. We conclude that the *dek1* locus is probably distal to *p1*. This prediction is consistent with more recent mapping data indicating that *dek1* is located 0.3 cM distal to *p1* (http://www.maizegdb.org/cgi-bin/displayposrecord.cgi?id=258944).

Substrate preferences for *Ac* transposition: Genetic studies have concluded that, in maize, transposition of simple *Ac* or *Ds* elements does not give rise to large deletions or other rearrangements at appreciable frequencies (Fedoroff *et al.* 1983; Fedoroff 1989; Kunze and Weil 2002); however, deletions, duplications, and chromosome breakage are readily produced through transposition reactions involving complex *Ac/Ds* elements. For example, the maize *doubleDs* element, which contains one *Ds* element inserted into a second identical *Ds* in opposite orientation, induces chromosome breakage at a high frequency (Courage *et al.* 1984; Doring *et al.* 1984, 1989). Molecular analyses have shown that *Ds*-induced breakage is associated with the formation of chromatid bridges by transposition reactions involving *Ds* termini located on sister chromatids (English *et al.* 1993; Weil and Wessler 1993). The deletions generated by SCT occur when the transposon termini reinsert into the chromosome from which they were excised (Figure 1).

What determines the competence of individual *Ac/Ds* termini to participate in transposition reactions? In maize, *Ac/Ds* transposes during or shortly after DNA replication, but only one of the *Ac/Ds* elements in the two sister chromatids is competent for transposition (chromatid selectivity) (Greenblatt and Brink 1962; Greenblatt 1984; Chen *et al.* 1987, 1992; Fedoroff 1989). Several lines of evidence show that the methylation status of *Ac* plays an important role in chromatid selectivity. Data from *in vitro* binding assays show that the Ac transposase binds to hemi-, holo-, and unmethylated *Ac* sequences with distinctly different affinities: strong binding occurs at hemi-methylated sites in which a particular strand is methylated, whereas sequences in which the opposite strand is methylated exhibit little binding (Kunze and Starlinger 1989). In addition, studies of *Ds* excision from extrachromosomal DNA introduced into petunia cells show that a *Ds* element hemi-methylated on one DNA strand has a 6.3-fold higher transposition frequency than an element methylated on the complementary strand (Ros and Kunze 2001). These and other data have led to a model for the control of transposition competence by differential binding of Ac transposase depending on methylation state (Wang *et al.* 1996). Similarly, the transposase of the prokaryotic *IS10* element binds to hemi- and holomethylated *IS10* ends with different affinities, thus determining which *IS10* copy is transposition competent after DNA replication (Roberts *et al.* 1985).

The methylation model for control of *Ac* transposition makes certain specific predictions regarding the transposition competence of the *Ac* and *fAc* termini in *p1-vv9D9A*. Immediately following replication of the *p1-vv9D9A* allele, the methylated DNA strand of the *fAc* element in one sister chromatid should be the same as that of the 3′-end of the *Ac* element in the other sister chromatid. Thus, the methylation hypothesis would predict that functional transposition complexes could involve (1) the 5′- and 3′-ends of the *Ac* element on one chromatid (standard transposition) or (2) the 3′-end of *fAc* and the 5′-end of *Ac* on different sister chromatids (sister-chromatid transposition). The former will result in *Ac* excision and generate a *P1-rr* allele with a *fAc* insertion, and the latter will generate deletions and corresponding inverted duplications. Indeed, both events are obtained, but the latter occurs at a lower frequency in the *p1-vv9D9A* allele (Zhang and Peterson 1999), whereas the methylation model predicts that transposition involving the 3′ *fAc* end and the 5′ *Ac* end on the same chromatid should not occur. If it did occur, then excision followed by reinsertion into the same chromosome would be predicted to generate inversions that include the *Ac* element and the genomic sequence to the reinsertion site (Figure 6). These inversions would disrupt *p1* function and could be detected in

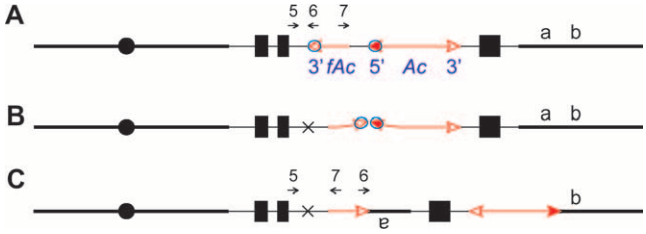

Figure 6.—Hypothetical transposition involving the *fAc* 3′-end and the *Ac* 5′-end on the same chromatid in *p1-vv9D9A* (symbols have the same meaning as in Figure 1). This type of transposition reaction would result in reorientation of the sequences hybridizing to oligonucleotide primers 6 (Ac-6) and 7 (Ac-7) (compare A and C) and thus could be detected by PCR. No such products were detected among 10 *p1-ww* alleles tested. See text for details. (A) *Ac* transposase binds to a *fAc* 3′-end and an *Ac* 5′-end in the same chromatid. (B) Cuts are made at the *Ac* and *fAc* termini; sequences at which the *Ac* and *fAc* termini were formerly inserted are joined together at the site marked by the **X**. (C) The excised transposon ends reinsert at a site between a and b. The DNA between *fAc* and the insertion site is inverted.

our screen for new *p1-ww* alleles. To determine whether such inversions might exist among our collection of 100 *p1-ww* alleles derived from *p1-vv9D9A*, we tested 10 *p1-ww* alleles among the 65 cases that did not show deletion of *p1* or *p2* in the initial PCR screen. DNA from these 10 *p1-ww* alleles was used in PCR with primer pairs p1-5/Ac-6 and p1-5/Ac-7. As shown in Figure 6, excision of the 3′ *fAc* and 5′ *Ac* termini from the same chromatid would result in a fusion of the 5′-end of *p1* intron 2 with the sequence adjacent to the 5′ breakpoint of the *fAc* element. If such a fusion occurred, the PCR product generated by p1-5/Ac-6 should disappear, and a new product from primers p1-5/Ac-7 should be formed. No such cases were identified among the 10 *p1-ww* alleles tested. These results suggest that when the *fAc* 3′-end and the *Ac* 5′-end are in direct orientation as in the *p1-vv9D9A* allele, the termini on sister chromatids are preferred transposition substrates. In contrast, we showed recently that when a *fAc* 3′-end and an *Ac* 5′-end are in reversed orientation (*i.e.*, pointing toward each other), then transposition involving termini on the same chromatid can occur, generating inversions and other products (Zhang and Peterson 2004). Taken together, these results support the model of Wang *et al.* (1996) in which assembly of a functional transposition complex requires the interaction of 5′ and 3′ transposon termini whose individual competence is determined by strand-specific methylation patterns.

Evidence from McClintock for *Ds*-induced deletions on chromosome 9s: Previous research indicates that the chromosome breaking (state I) *Dissociation* element originally identified by McClintock (1953) was a *doubleDs* element, which contains two copies of a simple *Ds* element, with one *Ds* inserted into the other in reversed orientation (Doring *et al.* 1984; English *et al.* 1993, 1995; Weil and Wessler 1993; Martinez-Ferez and Dooner 1997). Because *doubleDs* has two pairs of directly oriented 5′ and 3′ *Ds* termini pointing out from the element, hypothetically it could undergo SCT reactions to generate deletions on either side of the insertion, *i.e.*, in both the proximal and distal directions. Hence it was interesting to determine whether any evidence of deletions was previously reported by McClintock. In one experiment, McClintock isolated a number of mutant alleles derived from a chromosome 9s containing a state I (chromosome-breaking) *Ds* element and dominant alleles of the *colorless1* (*c1*), *shrunken1* (*sh1*), and *bronze1* (*bz1*) genes. The *c1* gene specifies purple aleurone pigmentation, and the functional *C1* allele is recessive to the dominant inhibitor allele *C1-I*. The *sh1* and *bz1* genes affect endosperm starch and aleurone color, respectively. The genes are linked in the order *c1*-(4 cM)-*sh1*-(2 cM)-*bz1*, and the *Ds* element was inserted in the *c1-sh1* interval, very close to *sh1* (McClintock 1953). SCT involving *Ds* could generate three classes of deletion mutants: proximal deletions that would remove *sh1* (*C1-I Ds* Δ*sh1 Bz1*) or both *sh1* and *bz1* (*C1-I Ds* Δ*sh1* Δ*bz1*), and distal deletions that remove *C1-I* (Δ*c1 Ds Sh1 Bz1*). Three stocks containing the chromosome of constitution *C1-I Ds Sh1 Bz1* were crossed with a *C1 sh1 bz1* stock, and progeny kernels were screened for the appearance of new mutants. Among an unspecified number of progeny kernels screened, McClintock reported finding 37 *C1-I Ds sh1 Bz1*, 12 *C1-I Ds sh1 bz1*, and 20 *c1 Ds Sh1 Bz1* cases. Several lines of evidence suggest that many of these mutants may have been SCT-induced deletions:

1. Some *C1-I Ds sh1 Bz1* mutants exhibited a pronounced decrease in crossover frequency between *sh1* and *bz1*; in one case, no crossovers between *sh1* and *bz1* were detected among 3156 tested gametes. This result is consistent with deletions that extend into the *sh1-bz1* interval. In contrast, the crossover frequency between *c1* and *sh1* was only slightly reduced. The observed small decrease in crossover frequency between *c1* and *sh1* could be expected as a consequence of the deletion of the short interval between *Ds* and *sh1*; the SCT-induced deletion should start from the *Ds* element and extend proximally to the *sh1* locus, and it was known that the *Ds* element is tightly linked to *sh1*.
2. Among 12 *C1-I Ds sh1 bz1* mutants, 6 showed decreased male and/or female transmission frequency, and 1 mutant was completely male and female sterile; similar transmission defects are a common feature of large deletions.
3. For all 20 cases showing losses of *C1-I*, no homozygous plants survived to maturity; all failed to germinate or died as seedlings. These results are consistent with deletion of essential genes, such as the *dek12* gene, which is located in the interval between *c1* and *sh1* (McClintock 1953; Neuffer *et al.* 1997).

McClintock (1953) reported that 10 of the 20 cases that showed losses of *C1-I* had lost the chromosome arm distal to the *Ds* element. For the remaining cases, however, there were no cytologically visible structural alterations in the short arm of chromosome 9.

The three lines of evidence described above are suggestive of the occurrence of deletions, but do not indicate how such deletions may have been generated. A possible clue to the mechanism is provided by McClintock (1953), who determined that *Ds* was still present on each of the mutant chromosomes she tested. This result is exactly what would be predicted for SCT of *doubleDs:* A chromosome-breaking *Ds* structure should be retained at the deletion junction, whereas deletions derived by standard transposition followed by excision of a macrotransposon as proposed by Page *et al.* (2004) would often not contain *Ds* in association with the deletion-bearing chromosome.

Generality and significance of SCT-induced deletions: The model for SCT is mechanistically very similar to that of standard cut-and-paste transposition, but the products are very different: standard transposition results in movement of the transposon to a new site in the genome, while the SCT reaction generates deletions, duplications, and, potentially, other rearrangements (Zhang and Peterson 1999). These products are generated due to the altered topology of the transposon termini: in both the *Ac/fAc* and *doubleDs* events discussed here, at least one pair of *Ac/Ds* 3′ and 5′ termini are in direct orientation relative to each other. We have recently shown that another type of unconventional transposition reaction can occur when *Ac* termini are oriented toward each other (reversed-ends transposition; Zhang and Peterson 2004). Reversed-ends transposition can generate deletions, inversions, and, potentially, other rearrangements.

The evidence presented above indicates that SCT has generated extensive and overlapping deletions at the maize *p1* locus on chromosome 1s and possibly also in the vicinity of the *sh1* locus on chromosome 9s. SCT-induced deletions have also been reported in transgenic tobacco (English *et al.* 1995). Thus, the SCT reaction can probably occur at any genomic location containing *Ac/Ds* termini in the appropriate orientation. Whether the SCT reaction can also occur with other members of the *hAT* transposon family remains to be determined.

The detection of deletions and chromosome breakage in the above studies was facilitated by the proximity of genes controlling nonessential, visible phenotypes. It should be possible to reproduce the SCT reaction using transgenes containing *Ac/Ds* termini with appropriate marker genes. This approach would enable the isolation of a deletion series, similar to that described here for *p1*, at any genomic location containing the transgene construct. The ability to generate numerous overlapping deletions in specific regions of plant genomes could be highly advantageous for genetic and physical mapping and for functional genomics research. Finally, the ability of SCT to generate large deletions may have provided a significant counterbalance to the tendency toward genome enlargement over evolutionary time (Bennetzen and Kellogg 1997).

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