Analysis of Extrachromosomal *Ac*/*Ds* Transposable Elements

Vera Gorbunova and Avraham A. Levy

Plant Sciences Department, The Weizmann Institute of Science, Rehovot, 76100 Israel

Manuscript received October 2, 1999
Accepted for publication January 12, 2000

ABSTRACT

The mechanism of transposition of the maize *Ac*/*Ds* elements is not well understood. The true transposition intermediates are not known and it has not been possible to distinguish between excision models involving 8-bp staggered cuts or 1-bp staggered cuts followed by hairpin formation. In this work, we have analyzed extrachromosomal excision products to gain insight into the excision mechanism. Plasmid rescue was used to demonstrate that *Ds* excision is associated with the formation of circular molecules. In addition, we present evidence for the formation of linear extrachromosomal species during *Ds* excision. Sequences found at the termini of circular and linear elements showed a broad range of nucleotide additions or deletions, suggesting that these species are not true intermediates. Additional nucleotides adjacent to the termini in extrachromosomal elements were compared to the sequence of the original donor site. This analysis showed that: (1) the first nucleotide adjacent to the transposon end was significantly more similar to the first nucleotide flanking the element in the donor site than to a random sequence and (2) the second and farther nucleotides did not resemble the donor site. The implications of these findings for excision models are discussed.

THE maize *Ac*/*Ds* transposable elements are thought to transpose by a cut-and-paste process involving a DNA intermediate and leaving a double-strand break in the host DNA. However, the exact transposition mechanism remains obscure. It is presumed that the target DNA is cleaved by 8-bp staggered cuts prior to insertion because of the presence of an 8-bp target site duplication on each side of the transposed *Ac*/*Ds* elements. The mode of transposon excision is less clear. Insight into the excision mechanism can be obtained from the analysis of "footprints" left at the donor site after excision. In typical footprints, the 8-bp target site duplications flanking the inserted element are left mostly intact except for 1 or 2 bp closest to the element; these bases are usually deleted, inverted, or replaced by their complement (Baran *et al.* 1992; Scott *et al.* 1996; Rinehart *et al.* 1997). Deletions of more than one or two bases also occur but are relatively rare. Two models were proposed to explain the formation of excision footprints. According to the first model (Figure 1A) (Saedler and Nevers 1985), *Ac* transposase makes 8-bp staggered nicks at the ends of the target site duplication, similarly to the type of cleavage catalyzed upon insertion that generates the 8-bp duplication. These cuts are speculated to leave 5′ overhanging ends (Saedler and Nevers 1985). Then repair synthesis initiated at the 3′ end of the host DNA fills the gaps utilizing the single-stranded fringes of the former duplication as templates. If the element is still present in the "excision complex," it has been proposed that polymerase may switch to the single strand attached to the transposon. This would generate excision footprints with predictable additional nucleotides in the middle of the host duplication. An alternative model (Figure 1B; Coen *et al.* 1989) proposes that excision is initiated by single-base-pair staggered nicks (instead of 8 bp) and that the broken termini of the donor site are covalently sealed into hairpins. These hairpins are then randomly nicked, and the rearranged termini are joined, giving rise to various footprints, similar to those predicted from the 8-bp overhang model (Saedler and Nevers 1985). Recent analysis of a large collection of excision footprints provided new insight into the effect of flanking sequences on the patterns of repair at the donor site after transposon excision and on the formation of predominant types of footprints (Scott *et al.* 1996; Rinehart *et al.* 1997). However, it still did not allow differentiation between the two excision models.

It is assumed that after cleavage, the single-stranded overhangs present on the excised transposon are degraded by exonuclease because *Ac*/*Ds* do not carry donor nucleotides to the new insertion site. In fact, the true intermediates formed in the course of *Ac* transposition are unknown. We have found previously (Gorbunova and Levy 1997a) that *Ac* transposition is accompanied by the formation of joined *Ac* ends, likely to correspond to circularized transposons. The sequence at the ends' junction is variable, containing small deletions and insertions. Circles with deleted transposon ends are not expected to successfully reintegrate. Circles having both transposon ends intact are also unable to reintegrate into the genome via the transposition pathway, suggesting that the circles are abortive trans-

Corresponding author: Avraham A. Levy, Plant Sciences Department, The Weizmann Institute of Science, Rehovot, 76100 Israel.
E-mail: avi.levy@weizmann.ac.il

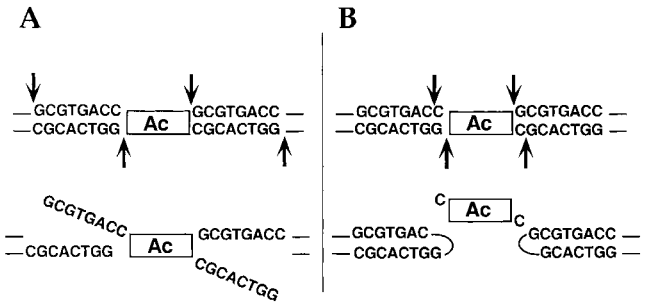

Figure 1.—Models for *Ac* excision. Transposase cuts are indicated by arrows. The sequence of 8-bp target site duplication flanking *Ac* element in *wx-m5* allele (and pAGS4411 construct) is shown as an example. (A) In the model proposed by Saedler and Nevers (1985), transposase introduces nicks staggered by 8 bp, in the same way as it cleaves during transposon insertion. (B) In the model proposed by Coen *et al.* (1989), transposase makes 1-bp staggered nicks during excision and leaves covalently closed (hairpin) DNA ends at the donor site.

position products (Gorbunova and Levy 1997a). It is possible that transposon circles are generated by host-mediated circularization of linear transposition intermediates because plants are capable of circularizing extrachromosomal linear DNA (Gorbunova and Levy 1997b).

The analysis of excision footprints does not enable us to distinguish between the excision via 8-bp or 1-bp staggered cuts. However, an insight into the excision mechanism can be provided by the analysis of ends junctions in *Ac* circles.

In this work, we report further analysis of transposon species formed during the course of transposition. We provide new direct evidence for the existence of transposon circles by rescuing circles containing a bacterial origin of replication in *Escherichia coli*. To learn about excision mechanism, a large collection of joined *Ac* ends was sequenced. Insertions found between the transposon ends were compared to the flanking regions at the donor site. We also sequenced the ends of extrachromosomal linear elements. As with the circles, the ends of linear elements were variable in structure, containing deletions and insertions, which suggests that they belong to elements that are unable to reintegrate. Analysis of the additional nucleotides found at the transposon termini in circular and linear transposons showed that the first nucleotide adjacent to the transposon end was statistically similar ($P < 0.001$) to the first nucleotide flanking the element in the donor site. The second (and farther) nucleotides, on the other hand, did not correspond to the donor site. The implications of these findings for models of *Ac* excision are discussed.

MATERIALS AND METHODS

Plasmids and transgenic plants: Transgenic tobacco plants were made by an Agrobacterium-mediated procedure (Horsch *et al.* 1985) using the following constructs: pAGS4411 (Dooner *et al.* 1991) and pAcGUS (Finnegan *et al.* 1989), which contain the full-length *Ac* element, were kindly provided by H. Dooner and E. Dennis, respectively; SLJ10512, expressing a stable *Ac* transposase protein (Bancroft *et al.* 1992), was kindly provided by J. Jones. A short *Ds* (2.8-kb) element was constructed to facilitate the separation of excised extrachromosomal linear elements from genomic DNA. This short *Ds* was cloned into the streptomycin excision cassette of binary vector pAGS3995 (Dooner *et al.* 1991), giving rise to construct pShortDs. The short *Ds* (2.8-kb) element was made by cloning the 2.3-kb *Cla*I-*Sac*II NPTII-containing fragment from pGA492 (An 1987) in between the termini of *Ac*, namely, the first 251 bp from *Ac* 5′ end and the last 265 bp from *Ac* 3′ end. pAGS4411, SLJ10512, and pShortDs were transformed into *Nicotiana tabacum* var. *Samsun*, and pAcGUS was transformed into *N. tabacum* var. *Xanthi*.

Transgenic tomato plants containing a *Ds* element with an *E. coli* origin of replication and chloramphenicol resistance (Ds-rescue) that can be used for plasmid rescue experiments (Rommens *et al.* 1992) were kindly provided by J. Hille, and transgenic tomato plants expressing a stable *Ac* transposase (st-Ac) under the 35S promoter control (Fedoroff and Smith 1993) were kindly provided by R. Meissner (Meissner *et al.* 1997). Crosses were done between transgenic plants carrying pShortDs and plants containing transposase (SLJ10512) to analyze the ends of extrachromosomal linear *Ds* elements. The progeny with actively transposing elements were selected on streptomycin-containing media as described (Jones *et al.* 1989).

DNA isolation: Genomic DNA for Southern blots and rescue experiments was prepared as described (Dellaporta *et al.* 1983). For sequencing free *Ac* ends the Dellaporta protocol was stopped at the stage where DNA is precipitated with isopropanol and resuspended in TE to minimize breakage of the DNA. Genomic DNA used for PCR assays was extracted by a minipreparation procedure as described (Bernatzky and Tanksley 1986).

Rescue of *Ds* circles: The F_1 plants of a cross between the transgenic tomato plants (Rommens *et al.* 1992) containing *Ds* with an *E. coli* origin of replication and chloramphenicol resistance and transgenic plants containing *Ac* transposase were used for plasmid rescue. These plants were tested by PCR for the presence of joined *Ds* ends (Gorbunova and Levy 1997a), which are associated with transposition activity and circle formation. Genomic DNA from the plants containing actively transposing *Ds* elements was transformed into Electromax DH10B competent cells (GIBCO BRL, Gaithersburg, MD), and chloramphenicol resistant clones were selected. Plasmid DNA was extracted from chloramphenicol-resistant clones and sequenced with the following primers: 5′GTTTTTTACCTCGGGTTCG for the 5′ *Ac* end and 5′GA AAATGAAAACGGTAGAGG for the 3′ *Ac* end.

Southern blot analysis: Southern blot analysis was done for tobacco and tomato genomic DNA, using probes that were radiolabeled by the random priming method (Feinberg and Vogelstein 1983). The blotting and hybridization was performed with MSI nylon membranes according to the manufacturer's protocol. The analysis of the inserts (filler DNA) found in rescued *Ds* circles, in between *Ds* ends, was done by hybridizing genomic DNA from wild-type tomato plants with probes consisting of the inserts in r3 and r5 clones. The filler DNA fragments used as probes were obtained by PCR amplification using the rescued plasmid DNA as template and primer pair 5′ACTAACAAAATCGGTTATACG + 5′GAAAATGAAAACG GTAGAGG corresponding to *Ac* termini.

To test whether the filler sequences in between *Ac* joined ends are related to the donor site, plants containing one single copy of *Ac* in the T-DNA, with no additional transposed *Ac*

elements, were analyzed by Southern blot. Genomic DNA from 30 plants transformed with pAcGUS was digested with *Hin*dIII plus *Eco*RV enzymes and hybridized with the probe to 5′*Ac* end. Then the membranes were stripped and hybridized with the probe to 3′*Ac* end. Sequencing of joined *Ac* ends was done only for the plants that showed a band of the size expected for *Ac*-T-DNA junction and the absence of other bands with both probes.

Sequencing joined *Ac* ends: PCR amplification, cloning, and sequencing of joined *Ac* ends was done as described (Gorbunova and Levy, 1997a). From each PCR reaction only one clone was sequenced to avoid identical clones that might be derived from the same *Ac* circle.

Sequencing free *Ac* ends: To facilitate separation of excised linear elements from genomic DNA the short *Ds* element was used. Genomic DNA was extracted from transgenic plants containing actively transposing *Ds* elements and from the plants with inactive *Ds* elements as a control. Approximately 50 μg of DNA was run on a 1% agarose gel, the 2.5-kb fraction was excised and gel-purified with a GeneClean kit (BIO101). This fraction was denatured by heating for 4 min at 95° and placed immediately on ice. The DNA was treated with 500 units of terminal transferase (Boehringer Mannheim, Indianapolis) in the presence of ddC according to manufacturer's instructions. This treatment creates 15–30 base long polyC tails at the 3′ DNA ends. Then DNA was phenol-extracted, ethanol-precipitated and one-tenth of it was used as template for one PCR reaction. After three cycles of amplification (1 min at 95°, 1 min at 55°, and 1 min at 72°) with 2.5 pmole of anchor-polyG primer, anchor and *Ac* specific primers were added and amplification was continued for an additional 30 cycles. One microliter from this PCR reaction was used as a template for the second round of amplification with anchor primer and nested *Ac* specific primer. The following oligonucleotides were used: anchor-polyG, (5′CTGGATGACAAGCAAACATTGGGGGGGGGGGGGGG); anchor, (5′CTGGATGACAAGCAAACATT); a specific primer for *Ac* 5′ end for the first round, (5′TCCCGAATTAGAAAATACGG); a nested specific primer for *Ac* 5′ end for the second round (5′GGTGAAACGGTCGGGAAACT); a specific primer for the 3′ end for the first round (5′CCCGTCCGATTTCGACTTTA); and a nested specific primer for the 3′ end for the second round (5′ACCGTATTTATCCCGTTCGT). PCR products were cloned into pGEM-T vector system (Promega, Madison, WI) and sequenced with the same primers as for the sequencing of rescued *Ds* circles.

RESULTS

Isolation of *Ac* circles by plasmid rescue: Using a PCR strategy, we have shown previously that *Ac* excision is accompanied by the formation of joined transposon ends that probably belong to circularized extrachromosomal elements (Gorbunova and Levy 1997a). To obtain direct evidence that transposon circles are present in the cell, we used a modified plasmid rescue technique. Plants transformed with a *Ds* element containing a bacterial origin of replication and a bacterial chloramphenicol resistance marker (Rommens *et al.* 1992) were crossed with transgenic plants that express *Ac* transposase under the control of 35S promoter (Meissner *et al.* 1997). *Ds* circles formed in these F_1 plants are expected to be functional *E. coli* plasmids. Therefore, undigested total genomic DNA from the F_1 plants was transformed into *E. coli*, and chloramphenicol-resistant colonies were selected. Joined *Ac* ends from seven rescued circles were sequenced, and the sequences are shown in Table 1. The junctions contained deletions or insertions and were similar to the junctions that we have detected previously by the PCR approach (Gorbunova and Levy 1997a).

Two of these junctions (r3 and r5) contained long insertions of 700 and 1000 bp, respectively. We hypothesized that these inserts were copied from genomic DNA during the end joining process, similarly to the formation of filler DNA associated with double-strand-break repair in plants (Gorbunova and Levy 1997b, 1999; Salomon and Puchta 1998). This was tested by hybridizing radiolabeled inserts to tomato genomic DNA digested by *Eco*RV, *Spe*I, and *Bgl*II restriction enzymes. The autoradiogram of the Southern blot is shown in Figure 2. For both inserts in r3 and r5 a hybridization signal was obtained with a pattern characteristic of a low or single copy sequence.

Based on the frequency of plasmid rescue, the amount of circles per cell can be roughly estimated. On average, transformation with 1 μg of tomato DNA from F_1 plants (Ds-rescue × St-Ac) yielded one colony of a transposon circle, while no colonies were obtained with a negative control consisting of tomato DNA from Ds-rescue plants that contain inactive *Ds* elements. We mixed pUC19 DNA with tomato genomic DNA, transformed it into *E. coli*, and determined the transformation efficiency, which was 10^7 cfu per microgram of pUC19 DNA. Assuming similar transformation rates for pUC19 and the rescued circles, we could calculate that to obtain one colony of *Ds* circle, there must be $\sim 3.4 \times 10^4$ transposon circles in 1 μg of genomic DNA. One microgram of tomato genomic DNA corresponds to 5×10^5 cells, assuming 2 pg of DNA per diploid genome (Arumuganathan and Earle 1991). Therefore, the concentration of *Ds* circles was estimated to be about one molecule per 15 cells.

Analysis of excised *Ac* ends: The analysis of excision footprints does not enable us to distinguish between the two current models for *Ac* excision (see Introduction and Figure 1), since the majority of observed footprints can be explained by either model. Therefore, to get more insight into the excision mechanism, we have analyzed the sequence at the ends of extrachromosomal *Ac*/*Ds* elements. Our assumption is as follows: if *Ac* is excised by 8-bp staggered cuts (Saedler and Nevers 1985) (Figure 1A), we expect that part or all of the eight nucleotides from the host duplication will be present in the extrachromosomal element. If, on the other hand, excision occurs by 1-bp staggered cuts (Coen *et al.* 1989) (Figure 1B), we expect that only the first nucleotide flanking the extrachromosomal element will correspond to the donor site. One important condition for this type of analysis is that the sequence of the donor site from where the element is excised should be known.

TABLE 1

Sequences of joined *Ds* ends obtained by plasmid rescue

No. of independent clones/name	Conceptual head-to-head joining of *Ac* ends		
	CGACCGTTTTCATCCCTA		*CAGGGATGAAAGTAGGA*
	Sequences of the PCR clones		
1/r3	CGACCGTTTTCATCCCTA	700 bp	CAGGGATGAAAGTAGGA
1	DEL of 21 bp		DEL of 30 bp
1	DEL of 23 bp TTTTCATC		CAGGGATGAAAGTAGGA
1	CGACCGTTTTCATCCCTA		AGGGATGAAAGTAGGA
1	CGACCGTTTTCATCCCTA	A	CAGGGATGAAAGTAGGA
1	CGACCGTTTTCATCCCTA	T	CAGGGATGAAAGTAGGA
1/r5	CGACCGTTTTCATCCCTA	1040 bp	CAGGGATGAAAGTAGGA

The sequence of the regions flanking *Ds* element is not shown, since the plants used for rescue experiments contained already secondary transposition events.

In a previous analysis (Gorbunova and Levy 1997a) we could not compare the insertions in between the ends to the donor site because the plants that we used contained several germinal secondary transposition events. In this work, the origin of the additional nucleotides adjacent to *Ac* ends in extrachromosomal elements was addressed in young primary transformant plants where the donor site is known for the majority of the excision events.

Joined *Ac* ends: We have shown above (Table 1 and Figure 2) that large insertions found at ends junction in rescued circular elements can be copied from a genomic template during the end joining process. However, the few nucleotides adjacent to element ends, in long or short inserts, may be excised together with the element, therefore originating from the donor site (Figure 1). This was tested on tobacco plants containing *Ac* within the T-DNA constructs, where the sequence of the flanking region is known. Two constructs with different *Ac* flanking regions were used to avoid any sequence bias.

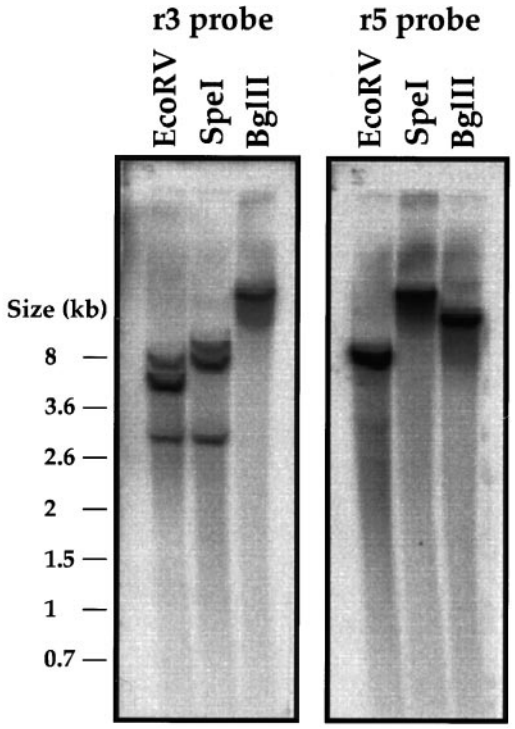

Figure 2.—Southern blot analysis of the two large inserts found between the element ends in *Ds* circles (r3 and r5 in Table 1). Wild-type genomic DNA from tomato was digested with *Eco*RV, *Spe*I, and *Bgl*II enzymes and hybridized with the probes consisting of the inserts in r3 and r5 clones.

Ac from wx-m7 allele: To ensure that most of the cells contain *Ac* in the original T-DNA, the period during which *Ac* could jump was minimized. Tobacco leaves were inoculated with Agrobacterium containing pAGS4411 (Dooner *et al.* 1991). This construct carries the *Ac* element that was originally subcloned from the *wx-m7* allele of maize together with ~30 nucleotides from *Waxy* on each side. When plantlets were formed, they were transferred onto selective medium that induces root formation. As soon as the first root appeared (which usually corresponds to the four-leaf stage), tissue was collected from each plantlet separately for DNA extraction. Therefore, every DNA sample represents an independent transformation event. By this approach, we expect that the majority of extrachromosomal *Ac*s are excised from the original T-DNA, rather than from a site formed by a secondary transposition event. Joined *Ac* ends were amplified with two nested pairs of primers (Figure 3A), and then PCR products were cloned and sequenced. Every DNA sample was amplified only once, and one clone from each reaction was sequenced to avoid sampling errors. DNA was extracted and amplified from 80 independent transformants. *Ac* circles were not found in all the plantlets, either because they were not transformed (very young plantlets may still escape the transformation selection) or because of the lack of transposition activity. The structure of the clones containing joined *Ac* ends is shown in Table 2. One clone had perfect joined ends, while all the others contained either

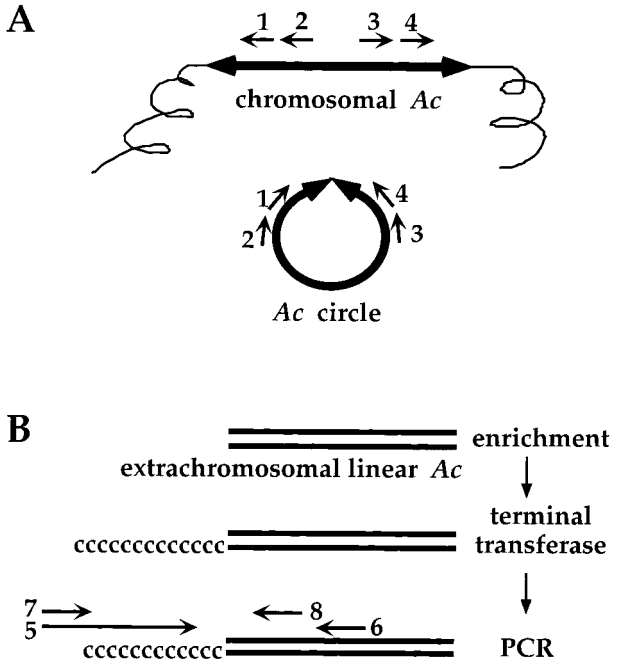

Figure 3.—Assays designed to amplify the ends of extrachromosomal *Ac* elements. PCR primers are shown as arrows. (A) A set of PCR primers designed to amplify joined *Ac* ends was used in two rounds of amplification on genomic DNA with primers 2 + 3 in the first and 1 + 4 in the second round. (B) To detect the ends of linear elements, genomic DNA was enriched for extrachromosomal linear elements by fractionation in agarose gel. PolyC tails were added to the 3′ DNA ends by terminal transferase. Then two rounds of PCR were performed; the first round consisted of three cycles of PCR with anchor-polyG(5)+*Ac* specific(6) primers, followed by 30 cycles with anchor(7)+*Ac* specific(6); the second round was performed with anchor(7)+nested *Ac* specific primer (8).

deletions or insertions. Clones with insertions were used for further analysis.

Ac from P-VV locus: T1 progeny of the primary transformant with pAcGUS that contains *Ac* from *P-VV* locus of maize were used (Finnegan *et al.* 1989). To minimize secondary transposition events, 30 plants were germinated and the presence of *Ac* in the original T-DNA was tested by Southern blots (data not shown). Out of 30 plants only one had *Ac* in the original position and did not contain new *Ac* elements. DNA from this plant was used as a template for 40 separate PCR reactions with primers shown in Figure 3. PCR products were cloned, and one clone from each PCR reaction was sequenced. The resulting clones are shown in Table 3. The overall structure of the clones from the *wx-m7* and *P-VV* alleles was similar; in many clones transposon ends were deleted, or insertions were found at the ends junction. Insertions were of different length; however, they could be readily divided into two groups. Short inserts, mostly of 1 to 3 bp, or long inserts (15 bp or more), interestingly, no inserts of 8 bp, and only one of 7 bp were found.

Sequencing of free *Ac* ends: The ends of extrachromosomal linear transposons have been characterized in the following way (Figure 3B): transgenic tobacco plants carrying the 2.8-kb-long *Ds* element (pShortDs) within the streptomycin excision cassette were crossed with the plants containing transposase in SLJ10512 construct (Bancroft *et al.* 1992). Genomic DNA from plants containing actively transposing *Ds* elements was run on an agarose gel and the 2.8-kb fraction was purified to enrich for linear elements. Then, this DNA was treated with terminal transferase in the presence of ddC to create a polyC tail at the end of the element and used as a template for PCR with *Ac* specific primer and polyG primer. As a negative control, the same treatments were performed on the DNA from plants lacking the transposase, containing inactive *Ds* elements. PCR products were obtained only from the plants containing actively transposing *Ds* elements but not from the negative control, indicating that free *Ac* ends do not arise from random DNA breakage but, rather, their occurrence is transposase dependent. The PCR products were cloned, and their sequences are shown in Table 4. Surprisingly, there was no predominant type among the sequences. In the majority of the sequences the transposon terminus was deleted, and the size of the deletion was from 5 to 50 bp. Interestingly, no deletions >50 bp were recovered, even though the assay could allow detection of bigger deletions. In several clones additional nucleotides were found at the transposon end.

Analysis of nucleotides flanking the ends of extrachromosomal elements: The correlation between the sequence of the nucleotides flanking the ends of extrachromosomal transposons and donor site was tested using all of the sequences containing insertions (Tables 2–4). Insertions of 1 bp were not taken into analysis for circles because they cannot be unambiguously assigned to one of the transposon ends. Nevertheless, it should be noted that out of the 10 cases of single-nucleotide insertions (Tables 2 and 3), 8 were identical to the nucleotide adjacent to one of the ends. Insertions adjacent to deleted ends were not considered either. Each transposon end was then considered separately, and the first nucleotide adjacent to this end was compared to the corresponding flanking region. With primary transformants of pAGS4411 (Table 2), in 19 out of 32 informative ends the first nucleotide of the insertion corresponded to the donor site. With progeny of a primary transformant containing a single copy T-DNA of pAcGUS (Table 3), in 15 out of 36 informative ends the first nucleotide of the insertion corresponded to the donor site. With F_1 seedlings of a cross between a pShortDs and transposase source from SLJ10512, only 7 informative ends were obtained and in 2 of these the first nucleotide of the insertion corresponded to the donor site. In total (Tables 2–4), in 36 (19 + 15 + 2) out of 75 informative ends chosen by the criteria described above, the first nucleotide of the insertion

TABLE 2

Sequences of joined *Ac* ends obtained by excision from pAGS4411 construct

	Flanking seq	**Ac**	Flanking seq
	.... GCGTGACC ← **CAG . . . . . CTA** → GCGTGACC		
	.... CGCACTGG ← **GTC . . . . . GAT** → CGCACTGG		
	Conceptual head-to-head joining of *Ac* ends		
No. of independent clones	*CGACCGTTTTCATCCCTA*		*CAGGGATGAAAGTAGGA*
	Sequences of the PCR clones		
1	CGACCGTTTTCATCCCTA	**G**	CAGGGATGAAAGTAGGA
5	CGACCGTTTTCATCCCTA	**C**	CAGGGATGAAAGTAGGA
1	CGACCGTTTTCA	**C**	CAGGGATGAAAGTAGGA
1	CGACCGTTTTCATCCCTA	T	CAGGGATGAAAGTAGGA
1	CGACCGTTTTCATCCCTA	T	TAGGA
5	CGACCGTTTTCATCCCTA	*GC*	CAGGGATGAAAGTAGGA
1	CGACCGTTTTCATCCCTA	*CT*	CAGGGATGAAAGTAGGA
1	CGACCGTTTTCATCCCTA	*AG*	CAGGGATGAAAGTAGGA
1	CGACCGT	CAC*	CAGGGATGAAAGTAGGA
1	CGACCGTTTTCATCCCTA	*ATC*	CAGGGATGAAAGTAGGA
1	CGACCGTTTTCATCCCTA	*CAG*	CAGGGATGAAAGTAGGA
1	CGACCGTTTTCATCCCTA	*GGTC*	CAGGGATGAAAGTAGGA
1	CGACCGTTTTCATCCCTA		CAGGGATGAAAGTAGGA
1	CGACCGTTTTCATCC	T	AAAGTAGGA
1	CGACCGT		A
1	CGACCGTTTTCATCC		GGATGAAAGTAGGA
2	CGACCGTTTTCA		GATGAAAGTAGGA
1	CGACCGTTTTCAT		CAGGGATGAAAGTAGGA
1	CGACCGTTTTCATCCCTA		AGGGATGAAAGTAGGA
1	CGACCGTTTTCATCCCT		AGGGATGAAAGTAGGA
1	CGACCG		CAGGGATGAAAGTAGGA
1	CGACCGTTTTCATCC		GGATGAAAGTAGGA
4	CGACCGTTTTCATCC		CAGGGATGAAAGTAGGA
1	Deletion of 19		AGGA
1	CGACCGTTTTCATCCCTA	*TA(29)GC*	CAGGGATGAAAGTAGGA
1	CGACCGTTTTCATCCCTA	*GA(20)AC	deletion of 18
1	CGACCGTTTTCATCCCTA	*TG(11)GG	AAGTAGGA
1	CGACCGTT	GG(19)AC*	CAGGGATGAAAGTAGGA
1	CGACCGTTTTCATCCCTA	*CC(66)TA	deletion of 31
1	CGACCGTTTTCATCC	GT(17)CC*	CAGGGATGAAAGTAGGA
1	CGACCGTTTTCATCCCTA	*GG(41)TA*	CAGGGATGAAAGTAGGA
1	CGACCGTTT	GA(64)AG*	CAGGGATGAAAGTAGGA
1	Deletion of 38	CA(34)AA*	CAGGGATGAAAGTAGGA

Nucleotides within the inserts that correspond to the flanking regions are shown as underlined boldface type. Numbers in brackets correspond to the number of base pairs.

*Informative ends used for statistical analysis.

corresponds to the donor site, whereas the expected number for a random nucleotide is 18.75 (=75/4). A chi-square test indicated that this deviation from the random expectation was highly significant ($P(\chi^2) < 0.001$). In other words, the first nucleotide of the insertion was significantly more similar to the first nucleotide flanking the element in the donor site than to a random sequence. Note that significant deviation from random expectation was also found when a chi-square test was done separately on data from Table 2 ($P(\chi^2) < 0.001$) or Table 3 ($P(\chi^2) < 0.025$) but not from Table 4 where the sample was too small to stand alone. A similar comparison was performed for the second and third nucleotides in sequences where the first nucleotide matched the donor site (the GC insertion from Table 2 was not taken into analysis, since the C nucleotide can be assigned to both ends). The sequence of the second and third nucleotides was unrelated to the donor site ($P(\chi^2) < 0.9$).

DISCUSSION

Circular and linear *Ac*/*Ds* elements in the cell: In a previous study we presented circumstantial evidence

TABLE 3

Sequences of joined *Ac* ends obtained by excision from pAcGUS construct

No. of independent clones	Flanking seq	**Ac**	Flanking seq
	AATAAATCT ← **CAG . . . . . CTA** ← CCCGTTCG TTATTTAGA ← **GTC . . . . . GAT** → GGGCAAGC		
	Conceptual head-to-head joining of *Ac* ends		
	CGACCGTTTTCATCCCTA		*CAGGGATGAAAGTAGGA*
	Sequences of the PCR clones		
1	CGACCGTTTTCATCCCTA	**C**	CAGGGATGAAAGTAGGA
2	CGACCGTTTTCATCCCTA	*__C__A*	CAGGGATGAAAGTAGGA
1	CGACCGTTTTCATCCCTA	*__CC__(33)GG*	CAGGGATGAAAGTAGGA
1	CGACCGTTTTCATCCCTA	*TC(53)__CT__*	CAGGGATGAAAGTAGGA
1	CGACCGTTTTCATCCCTA	*__C__A(314)T__T__*	CAGGGATGAAAGTAGGA
1	CGACCGTTTTCATCCCTA	*T(113)T__T__*	CAGGGATGAAAGTAGGA
1	CGACCGTTTTCATCCCTA	*__CT__*	CAGGGATGAAAGTAGGA
1	CGACCGTTTTCATCCCTA	*A__T__*	CAGGGATGAAAGTAGGA
1	CGACCGTTTTCATCCCTA	*TA(114)AC*	CAGGGATGAAAGTAGGA
1	CGACCGTTTTCATCCCTA	*GT(40)AG*	CAGGGATGAAAGTAGGA
1	CGACCGTTTTCATCCCTA	*TA(17)CA*	CAGGGATGAAAGTAGGA
1	CGACCGTTTTCATCCCTA	*GGAGC*	CAGGGATGAAAGTAGGA
1	CGACCGTTTTCATCCCTA	*TT(111)	GTAGGA
1	CGACCGTTTTCATCCCTA	*__CCC__(21)	GGGATGAAAGTAGGA
1	CGACCGTTTTCATCCCTA		GGATGAAAGTAGGA
1	CGACCGTTTTCATCCCTA	*__CC__	ATGAAAGTAGGA
1	CGACCGTTTTCATCCCTA	*TGA	AAAGTAGGA
1	CGACCGTTTTCATCC		CAGGGATGAAAGTAGGA
1	CGACCGTTTTC	(30)TC*	CAGGGATGAAAGTAGGA
1	CGACCGTTTTCATCC		CAGGGATGAAAGTAGGA
1	CGACCGTTTTCATC		CAGGGATGAAAGTAGGA
1	Deletion of 34 bp		CAGGGATGAAAGTAGGA
1	CGACCGTTTTCATCCCT	GG(41)AG*	CAGGGATGAAAGTAGGA
1	CGACCGTTTTCATCCCT	GT(12)T__T__*	CAGGGATGAAAGTAGGA
1	CGACCGTTTTCATCCCT	GCTCATG*	CAGGGATGAAAGTAGGA
1	CGACCGTTTTCATC	ACCA*	CAGGGATGAAAGTAGGA
1	Deletion of 18 bp		CAGGGATGAAAGTAGGA
1	CGACCGTTTTCATCCC		CAGGGATGAAAGTAGGA
1	CGACCGTTTTCATCCCT	GG*	CAGGGATGAAAGTAGGA
1	CGACCGTTTTCAT		CAGGGATGAAAGTAGGA
1	Deletion of 29 bp	(17)T__T__*	CAGGGATGAAAGTAGGA
1	Deletion of 31 bp	G__CT__*	CAGGGATGAAAGTAGGA
1	CGACCGTTTTCATCCCT		GGGATGAAAGTAGGA
1	CGACCGTTTTCATCC		AGGGATGAAAGTAGGA
1	C		AGGGATGAAAGTAGGA

Nucleotides within the inserts that correspond to the flanking regions are shown as underlined boldface type. Numbers in brackets correspond to the number of base pairs.

*Informative ends used for statistical analysis.

that transposon circles are formed during *Ac*/*Ds* excision (Gorbunova and Levy 1997a). Here, this work was extended by providing direct evidence, based on plasmid rescue, for the presence of transposon circles. The amount of circles was estimated at about one molecule per 15 cells. In addition, we have presented, for the first time, evidence for the presence of linear *Ds* elements. A detailed analysis of the sequences at the ends of circular and linear extrachromosomal elements is providing new insight into the fate of excised *Ac*/*Ds* elements and into the excision mechanism.

The majority of *Ac* circles contain deletions or insertions at the ends junction. Some transposon circles contain long insertions between their ends. These insertions were found to be derived from the host genome. The origin of these insertions is probably similar to that of filler DNA that is frequently formed at the sites of DNA double-strand-break repair in plants (Gorbunova and Levy 1997b; Rubin and Levy 1997; Salomon and Puchta 1998). Large insertions were shown by Southern blot analysis to be derived from tomato genomic DNA (Figure 2). Sequence analysis of one of the inser-

TABLE 4

Sequences at the ends of linear excised transposons

No. of independent clones	Flanking region in T-DNA	5′ *Ac* end
	GCGTGACC	CAGGGATGAAAGTAGGATGGGAAAATCCCGTACCGACCGTTATCGTATAACCGAT
	Additional nucleotides	Sequences of the clones
2		gAAAGTAGGATGGGAAAATCCCGTACCGACCGTTATCGTATAACCGAT
1		AGGATGGGAAAATCCCGTACCGACCGTTATCGTATAACCGAT
1		ggATGGGAAAATCCCGTACCGACCGTTATCGTATAACCGAT
1		AAAATCCCGTACCGACCGTTATCGTATAACCGAT
1		CCCGTACCGACCGTTATCGTATAACCGAT
1		gTACCGACCGTTATCGTATAACCGAT
1		ACCGACCGTTATCGTATAACCGAT
1		gACCGTTATCGTATAACCGAT
1		CGTTATCGTATAACCGAT
2		gTTATCGTATAACCGAT
1		ATAACCGAT
1		AACCGAT
2		CCGAT
1	CCCTAAGTGTATA	CAGGGATGAAAGTAGGATGGGAAAATCCCGTACCGACCGTTATCGTATAACCGAT
1	AGATACATGTGCT	CAGGGATGAAAGTAGGATGGGAAAATCCCGTACCGACCGTTATCGTATAACCGAT
1	ACGGCCGCGCT	CAGGGATGAAAGTAGGATGGGAAAATCCCGTACCGACCGTTATCGTATAACCGAT
1	CGACCCTGTATAGGGAG	CAGGGATGAAAGTAGGATGGGAAAATCCCGTACCGACCGTTATCGTATAACCGAT
1	gA**CC**	CAGGGATGAAAGTAGGATGGGAAAATCCCGTACCGACCGTTATCGTATAACCGAT
1	TTACCGACCGTTTTCATCCCTAGT**C**	CAGGGATGAAAGTAGGATGGGAAAATCCCGTACCGACCGTTATCGTATAACCGAT

No. of independent clones	3′ *Ac* end	Flanking region in T-DNA
	AAAATGAAAACGGTAGAGGTATTTTACCGACCGTTACCGACGTTTTCATCCCTA	**GCGTGACC**
	Sequences of the clones	Additional nucleotides
1	AAAATGAAAACGGTAGAGGTATTTTACCGACCGTTACCGACCGTTTTc	
2	AAAATGAAAACGGTAGAGGTATTTTACCGACCGTTAcc	
1	AAAATGAAAACGGTAGAGGTATTTTACCGACCGTTACCGACCGTTTTCATCCTA	ATCATCTGAT

Additional nucleotides corresponding to the flanking regions are underlined in boldface type. G or C nucleotides at the termini of clones are shown in lowercase, because they can be assigned to both the added Poly (C) tail and *Ac* sequences.

tions (318 bp) showed high homology to an unknown high-copy DNA from tobacco. Other insertions did not show significant homologies to sequences of known function.

We have identified extrachromosomal linear *Ds* elements. The occurrence of these elements, as expected, was transposase dependent. Transposon ends in these elements were variable containing deletions and additions. The additional bases at the ends of linear elements are probably the result of end elongation by some kind of repair synthesis using genomic DNA as template. Interestingly, the size of the deletions was always smaller than 50 bp, even though the assay system would allow detection of bigger deletions. This may correspond to the protecting effect of transposase binding this region (Kunze 1996).

Both transposon circles and extrachromosomal linear elements had similar modifications at the transposon termini. Similar rearrangements (deletions and insertions) were observed during the circularization of non-transposon linear extrachromosomal DNA (Gorbunova and Levy 1997b). This suggests that circles are derived by end joining of extrachromosomal linear elements done by the host DNA repair machinery. All the elements with deleted transposon termini are probably abortive products that were degraded by cellular exonucleases and lost the ability to reintegrate. Among the elements with additional bases at the ends, the size of the addition was variable. Among the identified sequences, there was no predominant type of product that could correspond to an active transposition intermediate. The phenomenon of "*Ac* loss," *i.e.*, excision without reintegration, was reported in several experimental systems and occurs in ~50% of the excisions (McClintock 1956; Greenblatt 1984; Dooner and Belachew 1989; Jones *et al.* 1990; Altmann *et al.* 1992; Dean *et al.* 1992). Exonuclease degradation and circularization of linear intermediates might represent one of the mechanisms that the host cell uses to reduce the number of transposons. The inability to detect a sufficient amount of a true transposition intermediate can be explained by the transient occurrence of such molecules in the cell. For bacterial transposons *Tn7* (Bainton *et al.* 1993) and phage *Mu* (Lavoie and Chaconas 1996), it has been shown that excision and reinsertion are coupled. For *Tn7* no cleavage is observed in the absence of target DNA and proteins responsible for target DNA interactions.

Mechanism(s) of *Ac* excision: As discussed above, the true *Ac* transposition intermediate remains elusive and the data described here relates to abortive excision products. In principle, there are maybe two different excision mechanisms: one for true and one for abortive transposition reactions. In this case our data would not provide insight into the true transposition mechanism but into the abortive one. Although this possibility cannot be excluded, the multiplicity of excision mechanisms has not been reported for any other transposable element. We therefore favor the following model, namely, that the *Ac*-encoded transposase catalyzes only one type of excision and that the excised element can either immediately reintegrate into a new site or be modified at its ends via deletion or addition of nucleotides followed eventually by end joining and circle formation. For *Ac* ends, these modifications can occur very rapidly because plants possess strong nuclease and end joining activities. Modified transposons might then accumulate in the cell, get lost in subsequent cell divisions, or recombine with the genome.

The analysis of extra nucleotides adjacent to the termini of extrachromosomal elements indicated that only the first but not the second nucleotide was significantly related to the donor site. This deviation from randomness was highly significant ($P(\chi^2) < 0.001$) when data from all the experiments were pooled (Tables 2–4): there was identity to the first nucleotide adjacent to *Ac* ends in 36 out of 75 ends, *i.e.*, almost twice more than expected for random addition of nucleotides (expected = 18.75 out of 75 ends). The deviation was the strongest when the biological material analyzed was as close as possible to the primary transformants (Table 2: 19 out of 32 ends, $P(\chi^2) < 0.001$). It was less strong but still significant (Table 3: 15 out of 36 ends, $P(\chi^2) < 0.025$) when DNA was extracted from older plants that had a higher probability to have accumulated secondary somatic transposition events into new unknown donor sites. We discuss below how this deviation from randomness fits into existing models of *Ac* excision.

If *Ac* is excised by 8-bp staggered cuts (Saedler and Nevers 1985) (Figure 1A), we expect that part or all of the eight nucleotides from the host duplication will be present in the extrachromosomal element. If, on the other hand, excision occurs according to the hairpin model (Coen *et al.* 1989) (Figure 1B), we expect to find only one nucleotide that corresponds to the nucleotide that immediately flanks the transposon in the extrachromosomal element. Additional nucleotides might then be added to the one adjacent to *Ac* by templated DNA synthesis. Data presented here showing that only the first but not the second nucleotide was significantly related to the donor site are best explained by the excision model with 1-bp staggered cuts at the ends of the element (Figure 1B, Coen *et al.* 1989). To explain these data with the 8-bp overhang model (Saedler and Nevers 1985) we would have to assume that there is protection of the first nucleotide flanking the transposon termini. We expect that if such protection occurs it would act beyond the first nucleotide. However, our findings of similar numbers of sequences with one, two, three, or zero additional nucleotides and also of deletions of various sizes going inside the terminal inverted repeats do not support the "protection" explanation.

In summary, although we cannot rule out the 8-bp overhang model or multiple excision pathways we favor

the hairpin model because it explains our data by making the least number of assumptions. From data presented here, one cannot infer the polarity of the cleavage and the location of the second nick. However, the position of the cleavage at the 3′ ends of transposon DNA appears to be conserved for all transposable elements whose transposition intermediates were studied, such as phage *Mu* (Lavoie and Chaconas 1996), *Tn7* (Bainton *et al.* 1991), *Tn10* (Benjamin and Kleckner 1992), *P* element (Beall and Rio 1997), and *Tc3* (Van Luenen and Colloms 1994). The 3′ DNA ends of the transposon DNA are always cut precisely at the transposon termini, while the 5′ ends are variable and can be inside the element, outside the element, or not at all, as in the case of phage *Mu*. Assuming that the same rules apply to *Ac* and that cleavage occurs one nucleotide outside of *Ac* termini, *Ac* would then be excised by a 1-bp staggered nick, leaving a 5′ overhang, with one nick being at the end of the element and another within the flanking DNA. This is in accordance with the model proposed by Coen *et al.* (1989) to explain footprint formation (see Introduction and Figure 1B). This model also proposes that after transposon excision, broken ends at the donor site form hairpins. Hairpins were shown to occur at coding DNA ends during V(D)J recombination (Roth *et al.* 1992). We have tried to detect hairpins at the donor site after *Ac* excision, using assays reported for V(D)J recombination. These attempts were not successful (data not shown), possibly because of very low abundance of intact broken donor molecules in the plant cells, compared to B cells undergoing V(D)J rearrangements. Recent findings showing that V(D)J recombination represents a transposition reaction (Agrawal *et al.* 1998; Hiom *et al.* 1998), where coding ends are analogous to the transposon donor site, make the Coen model very attractive. In addition, formation of hairpins was reported for *Tn10* transposition (Kennedy *et al.* 1998). There is also evidence suggesting that hairpins might be formed during transposition of other *Ac*-like elements: *Tam1*, *Tam3* (Coen *et al.* 1989), and *Ascot-1* (Colot *et al.* 1998). It is therefore possible that excision associated with hairpin formation is a widespread mechanism used by transposable elements.

We thank E. Eyal for help and discussions. V.G. was supported by a doctoral fellowship from the Schwartz Memorial Fund.

LITERATURE CITED

Agrawal, A., Q. M. Eastmann and D. G. Schatz, 1998 Transposition mediated by RAG1 and RAG2 and its implications for the evolution of the immune system. Nature **394:** 744–751.

Altmann, T., R. Schmidt and L. Willmitzer, 1992 Establishment of a gene tagging system in *Arabidopsis thaliana* based on the maize transposable element *Ac*. Theor. Appl. Genet. **84:** 371–383.

An, G., 1987 Binary Ti vectors for plant transformation and promoter analysis. Methods Enzymol. **153:** 292–305.

Arumuganathan, K., and E. D. Earle, 1991 Nuclear DNA content of some important plant species. Plant Mol. Biol. Rep. **9:** 208–218.

Bainton, R., P. Gamas and N. Craig, 1991 *Tn7* transposition *in vitro* proceeds through an excised transposon intermediate generated by staggered breaks in DNA. Cell **65:** 805–816.

Bainton, R. J., K. M. Kubo, J. Feng and N. Craig, 1993 *Tn7* transposition: target DNA recognition is mediated by multiple *Tn7*-encoded proteins in a purified *in vitro* system. Cell **72:** 931–943.

Bancroft, I., A. M. Bhatt, C. Sjodin, S. Scofield, J. D. G. Jones *et al.*, 1992 Development of an efficient two-element transposon tagging system in *Arabidopsis thaliana*. Mol. Gen. Genet. **233:** 449–461.

Baran, G., C. Echt, T. Bureau and S. Wessler, 1992 Molecular analysis of the maize *wx-B3* allele indicates that precise excision of the transposable *Ac* element is rare. Genetics **130:** 377–384.

Beall, E. L., and D. C. Rio, 1997 Drosophila P-element transposase is a novel site-specific endonuclease. Genes Dev. **11:** 2137–2151.

Benjamin, H. W., and N. Kleckner, 1992 Excision of *Tn10* from the donor site during transposition occurs by flush double-strand cleavages at the transposon termini. Proc. Natl. Acad. Sci. USA **89:** 4648–4652.

Bernatzky, R., and S. D. Tanksley, 1986 Methods for detection of single or low copy sequences in tomato on Southern blots. Plant Mol. Biol. Rep. **4:** 37.

Coen, E. S., T. P. Robbins, J. Almeida, A. Hudson and R. Carpenter, 1989 Consequences and mechanism of transposition in *Antirrhinum majus*, pp. 413–436 in *Mobile DNA*, edited by D. E. Berg and M. M. Howe. American Society for Microbiology, Washington, DC.

Colot, V., V. Haedens and J.-L. Rossignol, 1998 Extensive, nonrandom diversity of excision footprints generated by *Ds*-like transposon *Ascot-1* suggests new paralleles with V(D)J recombination. Mol. Cell. Biol. **18:** 4337–4346.

Dean, C., C. Sjodin, T. Page, J. Jones and C. Lister, 1992 Behaviour of the maize transposable element *Ac* in *Arabidopsis thaliana*. Plant J. **2:** 69–81.

Dellaporta, S. L., J. Wood and J. B. Hicks, 1983 A plant DNA minipreparation: Version II. Plant Mol. Biol. Rep. **1:** 19–21.

Dooner, H. K., and A. Belachew, 1989 Transposition pattern of the maize element *Ac* from the *bz-m2(Ac)* allele. Genetics **122:** 447–458.

Dooner, H. K., J. Keller, E. Harper and E. Ralston, 1991 Variable patterns of transposition of the maize element Activator in tobacco. Plant Cell **3:** 473–482.

Fedoroff, N. V., and D. L. Smith, 1993 A versatile system for detecting transposition in *Arabidopsis*. Plant J. **3:** 273–289.

Feinberg, A. P., and B. Vogelstein, 1983 A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132:** 6–13.

Finnegan, E. J., B. H. Taylor, S. Craig and E. S. Dennis, 1989 Transposable elements can be used to study cell lineages in transgenic plants. Plant Cell **1:** 757–764.

Gorbunova, V., and A. A. Levy, 1997a Circularized *Ac*/*Ds* transposons: formation, structure and fate. Genetics **145:** 1161–1169.

Gorbunova, V., and A. A. Levy, 1997b Non-homologous DNA end joining in plant cells is associated with deletions and filler DNA insertions. Nucleic Acids Res. **25:** 4650–4657.

Gorbunova, V., and A. A. Levy, 1999 How plants make ends meet: DNA double-strand break repair. Trends Plant Sci. **4:** 263–269.

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

Hiom, K., M. Melek and M. Gellert, 1998 DNA transposition by the RAG1 and RAG2 proteins: a possible source of oncogenic translocations. Cell **94:** 463–470.

Horsch, R. B., J. E. Fry, N. L. Hoffmann, D. Eichholtz, S. G. Rogers *et al.*, 1985 A general and simple method for transferring genes into plants. Science **227:** 1229–1231.

Jones, J. D. G., F. M. Carland, P. Maliga and H. K. Dooner, 1989 Visual detection of transposition of the maize element *Activator (Ac)* in tobacco seedlings. Science **244:** 204–207.

Jones, J. D. G., F. C. Carland, E. Lim, E. Ralston and H. K. Dooner, 1990 Preferential transposition of the maize element *Activator* to linked chromosomal locations in tobacco. Plant Cell **2:** 701–707.

Kennedy, A. K., A. Guhathakurta, N. Kleckner and D. Haniford, 1998 Tn10 transposition via a DNA hairpin intermediate. Cell **95:** 125–134.

Kunze, R., 1996 The maize transposable element *Activator (Ac)*, pp.

162–187 *Current Topics in Microbiology and Immunology: Transposable Elements*, edited by H. Saedler and A. Gierl. Springer-Verlag, Berlin.

Lavoie, B. D., and G. Chaconas, 1996 Transposition of phage Mu DNA, pp. 83–99, in *Current Topics in Microbiology and Immunology: Transposable Elements*, edited by H. Saedler and A. Gieh. Springer-Verlag, Berlin.

McClintock, B., 1956 Mutations in maize. Carnegie Institution, Washington Yearbook **55:** 323–332.

Meissner, R., Y. Jacobson, S. Melamed, S. Levyatuv, G. Shalev *et al.*, 1997 A new model system for Tomato Genetics. Plant J. **12:** 1465–1472.

Rinehart, T. A., C. Dean and C. Weil, 1997 Comparative analysis of non-random DNA repair following *Ac* transposon excision in maize and *Arabidopsis*. Plant J. **12:** 1419–1427.

Rommens, C. M. T., G. N. Rudenko, P. P. Dijkwel, M. J. J. Vanhaaren, P. B. F. Ouwerkerk *et al.*, 1992 Characterization of the Ac/Ds behaviour in transgenic tomato plants using plasmid rescue. Plant Mol. Biol. **20:** 61–70.

Roth, D., J. Menetski, P. Nakajima, M. Bosma and M. Gellert, 1992 V(D)J recombination: broken DNA molecules with covalently sealed (hairpin) coding ends in scid mouse thymocytes. Cell **70:** 983–991.

Rubin, E., and A. A. Levy, 1997 Abortive gap repair: the underlying mechanism for *Ds* elements formation. Mol. Cell. Biol. **17:** 6294–6302.

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

Salomon, S., and H. Puchta, 1998 Capture of genomic and T-DNA sequences during double-strand break repair in somatic plant cells. EMBO J. **17:** 6086–6095.

Scott, L., D. Lafoe and C. Weil, 1996 Adjacent sequences influence DNA repair accompanying transposon excision in maize. Genetics **142:** 237–246.

Van Luenen, H. G. A. M., and S. D. Colloms, 1994 The mechanism of transposition of Tc3 in *C. elegans*. Cell **79:** 293–301.

Communicating editor: V. Sundaresan