Sexual Transmission of Transposed *Activator* Elements in Transgenic Tomatoes

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Manuscript received November 7, 1988
Accepted for publication June 6, 1989

ABSTRACT

The transmission of transposed *Ac* elements in progeny derived by self-pollination of ten transformed tomato plants has been examined by Southern hybridization analysis. We show that six of these primary transformants have transmitted a transposed *Ac* to at least one progeny. One of the families was segregating for at least two different insertion events. In five of ten families, progeny were detected that contained a transposed *Ac* but no donor T-DNA sequences, indicating that a recombination event occurred between the original and new *Ac* insertion site. Somatic transposition of *Ac* as late as the R2 generation is evidenced. One family contained an empty donor site fragment but *Ac* was not detected in either the parent or progeny, indicating *Ac* was lost in this population early in regeneration. While four of ten families were segregating for aberrant phenotypes, there was no evidence that the mutated gene was linked to a transposed *Ac*.

UNTIL recently, the detailed study of plant transposons has been limited to those species which harbor characterized endogenous transposons. For a number of reasons, the characterization of transposable elements transformed into unrelated plant species is of interest (BAKER *et al.* 1986; VAN SLUYS, TEMPE and FEDEROFF 1987; KNAPP *et al.* 1988; YODER *et al.* 1988). Because endogenous elements are usually present in high genomic copy number (GEISER *et al.* 1982; UPADHYAYA *et al.* 1985), it can be difficult to follow the behavior of a particular element. Also, the lack of a routine transformation and regeneration system in many species harboring characterized transposons precludes the use of reverse genetics to study the biological effects of *in vitro* modifications to known elements. Third, since transposable elements are valuable tools for isolating genes encoding unknown gene products, the introduction of elements into heterologous species promises a powerful approach to molecularly access germplasm in these species. We have chosen *Lycopersicon esculentum* as the host plant in which to study the maize transposon family *Ac* and *Ds* because of the advanced state of its genetics (RICK and YODER 1988), the absence of *Ac*-hybridizing sequences endogenous to tomato (YODER *et al.* 1988), and its amenability to Agrobacterium-mediated gene transfer techniques (MCCORMICK *et al.* 1986).

It is clear from a number of studies that *Ac* will transpose at both early and late stages in the regeneration of transformed plants. In 10–12-day-old tobacco calli, BAKER *et al.* (1987) found that *Ac* had excised from at least one copy of the donor T-DNA in 13–40% of transformed calli. A similar observation was made in transformed carrot root cultures, where 28% of the cultures showed evidence of *Ac* excision (VAN SLUYS, TEMPE and FEDEROFF 1987). Transposition of *Ac* in later stages of development was detected by Southern hybridization with DNA isolated from different leaves of the same transformant (YODER *et al.* 1988). While the high level of *Ac* activity in somatic tissue of primary transformants is encouraging for using transposon mutagenesis in heterologous systems, it is critical to determine whether transposed elements are sexually transmitted to progeny where mutant alleles can be scored. The sexual transmission of a transposed element requires that the transposition event occur in a cell lineage which eventually develops into either male or female gametophytes. Because germ lineages are not established early in the development of plants, a transposition event may sector into both somatic and germ cells of a given plant. Sexually transmitted transpositions are termed germinal events since at least some of the cells containing the transposed element develop into gametophytes. This definition does not imply that transposition occurred at a given developmental stage; only that some of the cells in the affected cell lineage develop into gametes.

A second factor critical to the success of transposon tagging is the correlation of a particular insertion and the presence of a mutant allele. Tight linkage of a transposable element and mutant allele is initial evidence that the mutation results from the insertion of that element; further studies with revertant or inde-

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pendently derived alleles is necessary to confirm the causality. One difficulty which arises with transposon tagging using foreign elements introduced into plants via transformation is the high mutation rate inherent to the regeneration process. For example in tomato, upwards of 5% of the progeny of regenerated plants may have somaclonal mutations (EVANS 1988).

In the present study, we have used Southern hybridization to characterize the transmission of transposed *Ac* elements from the R_0 to the R_1 generation of transgenic tomato plants. Progeny obtained from selfing ten primary transformants have been analyzed; four of these ten families were segregating for abnormal phenotypes, the other six families appeared normal throughout their growth. Using three criteria, we distinguished forward transposition events which were genetically transmitted to progeny from those that occurred somatically in the R_1. In six out of ten primary transformants, a transposed *Ac* was genetically transmitted to at least one of the progeny examined, indicating a transposition event had occurred in a cell lineage destined, at least in part, to produce gametes. In total, nearly 30% of the progeny of primary transformants inherited a transposed *Ac* from their parent. In five of the ten families, at least one progeny contained a transposed *Ac* but lacked the donor T-DNA, indicating meiotic recombination between the original site of the element and its new position. Further analysis of progeny from plants which contained a single transposed element indicated the element is capable of continual transpositions for at least three generations following transformation.

While a number of related progeny contained the same forward insertion, there was no correlation between the mutations observed in the four selected families and a particular *Ac* insertion. Indeed in one case, a family segregating for a somatically variegated phenotype contained no *Ac* at all, indicating the element was lost during regeneration of the primary plant. We conclude that the mutations obtained in these four transformants arose from mechanisms other than the insertion of the introduced *Ac*. Because we observed no difference in *Ac* behavior between the mutant and normal phenotypes, results from the different families are compiled in this paper.

MATERIALS AND METHODS

Plant material: The tomato cultivar VF36 was transformed with pMAC, a derivative of pMON200 (FRALEY *et al.* 1985) bearing the maize transposon *Ac7* (Figure 1). The construction of pMAC and the transformation of VF36 was previously described (YODER *et al.* 1988). Following the convention proposed by CHALEFF (1981), primary transformants are called the R_0; progeny which result from selfing R_0 plants are R_1.

Self seed was collected from 30 primary transformants and from 20 to 100 seed per family were sown in the greenhouse. Progeny were visually scored for phenotypic

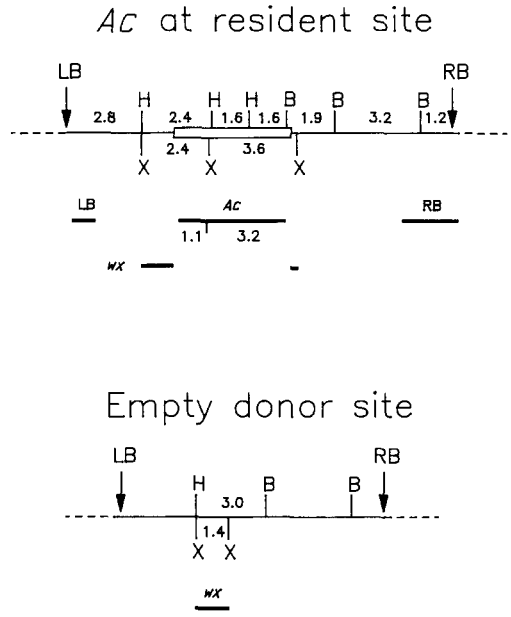

FIGURE 1.—Restriction map of integrated pMAC. The restriction map of a single copy of the transforming vector pMAC integrated into the tomato genome is given. The first map diagrams an untransposed *Ac* remaining at its resident position in the vector. The second map diagrams the empty donor site generated by excision of *Ac* from pMAC. The left (LB) and right (RB) borders of the T-DNA are depicted at the arrows. The fragment sizes generated upon digestion with *Xba*I (X) or double digestion with *Bam*HI (B) and *Hin*dIII (H) are given in kb. The two *Ac*-specific probes, 1.1 and 3.2, the two T-DNA border-specific probes, LB and RB, and the *wx*-specific probe are shown as thick lines below the restriction fragments homologous to these probes.

aberrations and four families with interesting phenotypic variants were selected for the molecular analysis described here. These four lines are: 88-01, segregating for a round leaf shape (*rlm*); 88-08, segregating for a variegated leaf chlorosis (*var*); 88-14, for a lethal albino mutation (*lab*) and 88-94, containing a mutation resulting in both chlorosis of the leaves as well as an entire leaf shape (*bzr*). Three of these mutants (88-01, 88-08, 88-14) segregated in the R_1 progeny in ratios consistent with being simple monogenic recessive mutations. The fourth, 88-94, appeared only one time in about fifty progeny seedlings. A more complete genetic analysis of these and other mutations obtained from this screening will be presented elsewhere (J. I. YODER *et al.*, in preparation). In order to get a general picture of the behavior of *Ac* in transgenic progeny, we also characterized the segregation of *Ac* in six families which appeared phenotypically normal. These ten families are listed in Table 1.

Southern hybridizations: Leaf tissue was harvested from 6 to 17 progeny of the ten selected primary transformants. Genomic DNA was isolated from frozen tissue by the CTAB method described by BERNATZKY and TANKSLEY (1986). Ten micrograms of genomic DNA were digested with either *Bam*HI and *Hin*dIII simultaneously or with *Xba*I. The digested samples were separated electrophoretically on 0.8% agarose gels and transferred onto Zeta-Probe membrane filters (Bio-Rad) according to the manufacturer's recommendations. Filters were baked in a vacuum oven at 80° for 1 to 3 hr. Prehybridizations (4 hr) and hybridizations (16 hr) were conducted at 42° in 5 × SSC, 10 × Denhardt's solution, 50 mM $NaPO_4$ (pH 7.0), 10% dextran sulfate, 1% SDS, 500 μg/ml denatured salmon sperm DNA and 50% formamide. Filters were washed at 65° in 0.2 × SSC, 1%

TABLE 1

Summary of R_1 Southern blot data

Family	Phenotype	No. progeny	No. copies T-DNA	Segregation				Progeny inheriting a transposed *Ac*[a]
				Ac T-DNA	Ac —	— T-DNA	— —	
88-01	rlm	17	1	12	2	3	0	12[b]
88-04	wt	6	1	4	0	2	0	0
88-05	wt	6	1	4	1	1	0	1
88-08	var	12	>2	0	0	9	3	0
88-09	wt	6	>2	6	0	0	0	0
88-10	wt	6	1	2	0	1	3	2
88-11	wt	6	1	5	0	0	1	0
88-12	wt	6	>1	2	1	2	1	1
88-14	lab	12	4	10	1	0	1	5
88-94	bzr	13	1	7	1	3	2	5
Total		90		52	6	21	11	26

[a] Using the three criteria described in RESULTS.
[b] See RESULTS.

SDS, and 0.1% Na-pyrophosphate. Before reprobing, filters were stripped with two 15-min washes at 95° using the wash solution.

A 4.3-kb *Cla*I-*Bam*HI fragment from pJAC-D (YODER *et al.* 1988) was used as the *Ac*-specific probe. Digestion of this fragment with *Xba*I results in two fragments, 1.1 kb and 3.2 kb, which were used separately to distinguish individual *Ac* insertions on *Xba*I blots. DNA for the *wx*-specific probe was isolated as a 3.2-kb *Sal*I fragment from pSALC (generously provided by S. WESSLER, Georgia). A *Hpa*I-*Ava*I double digest of pMON200 (FRALEY *et al.* 1985) yielded a 2.1-kb fragment used as a T-DNA right border probe. A *Sac*I digestion of the same plasmid produced an 800-bp left border-specific probe. The location of these probes with respect to an integrated pMAC vector is shown in Figure 1. DNA fragments were electrophoresed twice through agarose gels, the second separation being done in low melting point agarose. The fragments were labeled with ^{32}P by the random primer method (FEINBERG and VOGELSTEIN 1983) without prior extraction from agarose using a commercial kit (Amersham).

RESULTS

Strategy for examining *Ac* segregation in progeny: We used Southern hybridization to characterize the segregation of transposed *Ac* elements in selected R_1 populations. Genomic DNA was isolated from 6 to 17 progeny of each family and digested simultaneously with *Bam*HI and *Hin*dIII. The 4.3-kb *Ac*-specific probe shown in Figure 1 was hybridized to a Southern blot prepared from the double-digested DNA. An integrated pMAC plasmid produces three *Ac*-homologous fragments under these conditions: two internal 1.6-kb fragments and a 2.4-kb fragment containing one end of *Ac* together with flanking *wx* sequences (Figure 1). The 1.6-kb bands are indicative of an *Ac* element being present at any location in the genome. The presence of the 2.4-kb band indicates that at least one *Ac* remains at its resident position in the T-DNA in the tissue from which DNA was isolated. The same 2.4-kb resident band is detected using the *wx*-specific probe (Figure 1). The small amount of *wx* sequences on the right side of *Ac* results in a 1.9-kb band detected only with prolonged exposures.

When *Ac* transposes to a new chromosomal location, the 2.4-kb band will disappear upon probing with either *Ac* or *wx* sequences. Using the *Ac* probe, a new band of an unpredictable size will result for each *Ac* insertion. Cosegregation of an *Ac* insertion band with a mutant phenotype in the R_1 would suggest close linkage of the transposed element and the mutated gene. Probing with *wx*-specific sequences, excision of *Ac* results in the appearance of an empty donor site band of 3.0 kb.

Ac insertion bands can result either from somatic transposition within the plant examined or from the inheritance of elements which had transposed in the parent. We have used three criteria to distinguish between these two possibilities. First, if parent and offspring share a common transposed *Ac* insertion site band, we conclude that this transposed *Ac* was sexually transmitted from parent to progeny. To rule out the possibility of coincidental comigration of these bands, two different digestions were performed: first a *Hin*dIII-*Bam*HI double digestion as previously described and then a *Xba*I digestion. This last enzyme cuts only once in *Ac* (Figure 1). Therefore the 4.3-kb *Ac* probe detects two bands for each *Ac* insertion. If parent and offspring have identical bands on both blots, this transposed *Ac* was considered to have been inherited from the parent.

Our second criterion exploits the fact that if transposition occurs in a cell from which a number of gametes are eventually derived then similarly migrating insertion bands should be observed in different R_1 progeny. However, if progeny have a number of *Ac*-specific bands because the element is somatically active, bands that migrate similarly in siblings may

also reflect coincidental comigration of somatic transpositions. Therefore, here again, Southern hybridizations were performed following digestion of the DNA with *Hin*dIII-*Bam*HI and *Xba*I. If the same siblings had comigrating bands in both *Bam*HI-*Hin*dIII and *Xba*I blots, the comigrating bands were considered to result from the genetic transmission of transposed *Ac*s. An alternative approach was to sequentially probe an *Xba*I digest with each half of *Ac* (1.1- and 3.2-kb *Xba*I fragments) to identify which two bands were derived from the same transposed *Ac*. Therefore, comigration of *Ac* insertion bands in siblings in both the *Bam*HI-*Hin*dIII and *Xba*I blots, or in the *Xba*I blot when different *Ac* probes were used, indicated genetic segregation of a transposed element.

Third, the presence of a transposed *Ac* in a progeny devoid of T-DNA sequences indicates that a transposed *Ac* was inherited from the parent. Such occurrences require that *Ac* transpose away from the T-DNA locus in the parent. This then allows recombination and assortment of the two loci. Therefore an *Ac* inherited without T-DNA had to have transposed first in the parent. To score the progeny for the presence of *Ac* and T-DNA sequences, a *Hin*dIII-*Bam*HI blot was probed sequentially with the 4.3-kb *Ac* probe and the *wx* probe. This digestion allowed the detection of *Ac* sequences irrespective of their location thanks to the internal 1.6-kb doublet which hybridizes with the *Ac* probe. The *wx* probe detected either a 2.4-kb resident band or a 3.0-kb empty donor site. The blots were further probed with T-DNA right and left border-specific probes to determine the number of copies of pMAC in the transformants. Every progeny which contained *wx* sequences, either as a resident or an empty donor site fragment, also contained T-DNA border sequences. Therefore, the presence of the *wx* revertant band, pMAC resident band, or T-DNA borders could be used to identify the T-DNA insertion locus.

Genetic transmission of the same transposed *Ac* in related R_1 siblings: One progeny plant from the 88-94 family had yellow-green leaves which had a shape similar to that of the *entire* mutation (RICK and BUTLER 1956). All the progeny obtained from selfing this plant had the variant phenotype. The detection of one mutant in a population of about fifty seedlings suggested the mutation event occurred late in the development of the primary transformant so this variant was selected for further molecular analysis. Contamination of the seed lot was ruled out because its phenotype is unique among the lines collected at Davis (C. RICK personal communication).

DNA was isolated from the mutant individual, 88-94 A, and from twelve wild-type plants, 88-94 B-M. When the genomic DNA was digested with *Bam*HI-*Hin*dIII and probed with *Ac*, six progeny (C, D, E, F, H and M) contained a new *Ac*-specific band of 1.8 kb (Figure 2). When the same DNA was digested with *Xba*I and probed with *Ac*, five progeny (C, D, E, F and M), contained identically migrating bands of 1.9 kb and 8.0 kb (Figure 3). One progeny, H, had the common 1.8-kb insertion fragment in the *Bam*HI-*Hin*dIII digest, the common 1.9-kb band in the *Xba*I digest, but the larger fragment in the *Xba*I digest was reduced in size relative to the other progeny. This suggests a rearrangement or deletion in the plant DNA which flanks the *Bam*HI end and possibly in the *Bam*HI end of *Ac* itself.

Using the criteria discussed above, we estimated that at least five of the 13 progeny from this family had inherited a transposed *Ac* at the same new chromosomal position. However, plant A contained neither the segregating *Ac* insertion bands nor the 1.6-kb internal *Ac* fragments. In order to determine whether plants which contained the segregating transposed *Ac* (C, D, E, F and M) were heterozygous for the mutation, self seed from these R_1s was collected and from 20 to 50 were sown and scored. All of these R_2 progeny were normal. Taken together, these results ruled out the possibility that the transposed *Ac* which was segregating in this population marked the mutant locus.

In order to determine whether there was linkage between the T-DNA insertion and the mutation, the *Bam*HI-*Hin*dIII blot of 88-94 progeny was probed with *wx*. A 3.0-kb *wx*-specific band corresponding to the empty donor site was detected in ten of 13 progeny, consistent with a single insertion locus. This was verified by probing with right and left T-DNA border-specific probes; only one copy of pMAC had inserted into the genome at transformation (Figure 4). Progeny plant A, the mutant, was one of the three plants which did not contain a T-DNA insertion, indicating that the T-DNA was not marking the locus. This was further verified in the observation that self progeny from plants C, D, E and M did not transmit the mutant phenotype even though they contained the single T-DNA insertion.

Of additional interest was plant F which had *Ac* sequences but no *wx* or T-DNA sequences. The pattern observed in this plant must have arisen from meiotic recombination between the transposed *Ac* and the donor pMAC plasmid. The frequency of this event in the nine other families examined is described later.

Genetic transmission of a transposed *Ac* to siblings was also observed in a phenotypically normal family. In the family 88-10, two progeny, A and D, had the same 1.2-kb *Ac*-specific band in the *Hin*dIII-*Bam*HI digest. These same two plants also showed common bands of 4.8 and 8.5 kb in the *Xba*I digest when probed with *Ac* (Figure 5). In all, three progeny sets (88-10, 88-14 and 88-94) were ascertained as having

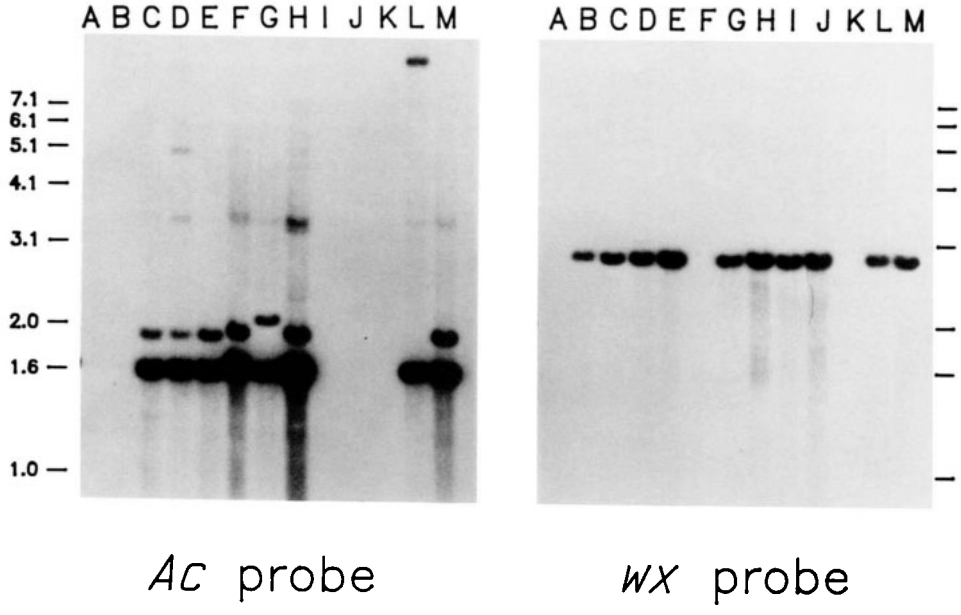

FIGURE 2.—*Bam*HI-*Hin*dIII double digest of 88-94 progeny. DNA from 13 R_1 siblings (A through M) was digested with both *Bam*HI and *Hin*dIII, electrophoretically separated on agarose gels, and blotted onto nylon membranes. The autoradiogram following probing with the 4.3-kb *Ac* probe is shown on the left. The 1.6-kb band segregating in this population is a doublet produced by fragments internal to *Ac*. The 1.8-kb band in plants C, D, E, F and M corresponds to the integration site of a germinally transposed *Ac*. The filter was then stripped and reprobed with the *wx*-specific probe as shown on the autoradiogram on the right. The segregating 3.0-kb band represents the empty donor site produced when *Ac* is excised from pMAC.

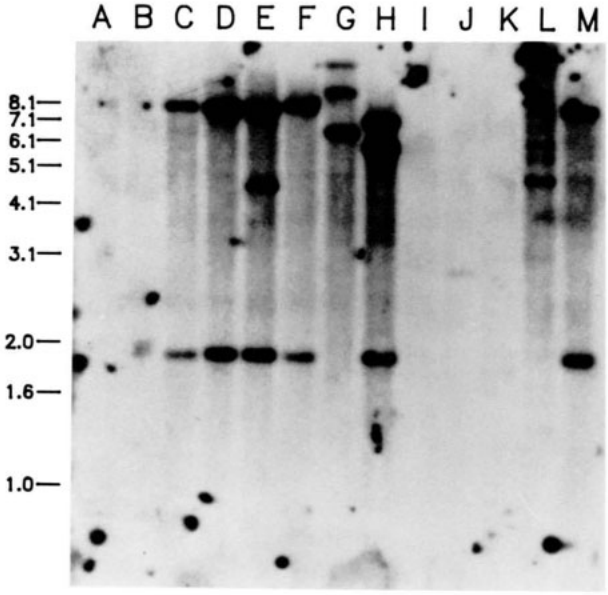

FIGURE 3.—*Xba*I digestion of 88-94 progeny. The 88-94 progeny DNAs were digested with *Xba*I and probed with the 4.3-kb *Ac* probe. The identically migrating bands in progeny C, D, E, F and M confirm that a germinally transposed *Ac* was inherited by these progeny.

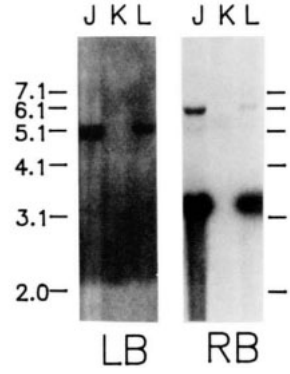

FIGURE 4.—T-DNA copy number determinations. The *Bam*HI-*Hin*dIII blot of 88-94 progeny was probed with the T-DNA left border (LB) and right border (RB)-specific probes. Three (J, K, L) of the thirteen progeny are shown. Single T-DNA insertion bands of 5.1 kb for the LB probe and 6.1 kb for the RB probe were detected. The 3.2-kb band using the RB probe is an internal T-DNA fragment produced by *Bam*HI sites internal to the right border. Progeny K is one of the three progeny which were not segregating for a T-DNA insertion.

inherited a transposed *Ac* using this criterion.

Sexual transmission of two different *Ac* insertions: Progeny from family 88-14 segregated for an albino mutation that was lethal before development of the first leaves, therefore the mutation was maintained as a heterozygote. Leaves were collected and DNA prepared from 12 young normal R_1 plants. The plants were then grown to maturity, self pollinated and seed collected. R_2 seed was sown and the populations scored for the albino trait. The mutation segregated in a manner consistent with the mutation being a monogenic recessive character (Table 2). By this analysis, we determined that R_1 plants 88-14 D, H, I, J and L were heterozygous for the mutation.

We digested 88-14 progeny DNA with *Xba*I and probed sequentially with the 1.1-kb and the 3.2-kb *Ac* probes (Figure 1). Fragments of 2.4 and 3.6 kb predicted for an untransposed, resident *Ac* were detected in the parent P as well as in progeny A, B, C, D, E, F, G, H, K and L (Figure 6). The intensity of these bands suggested that more than one copy of the resident pMAC molecule was present in some of the plants. This was consistent with T-DNA border analysis that indicated four copies of pMAC had inserted into the genome at transformation (not shown). In addition to the resident pMAC molecule, the parental DNA also contained a band of 1.5 kb when the 1.1-kb *Ac* probe was used and 20 kb with the 3.2-kb *Ac* probe. These bands must have resulted from somatic transposition

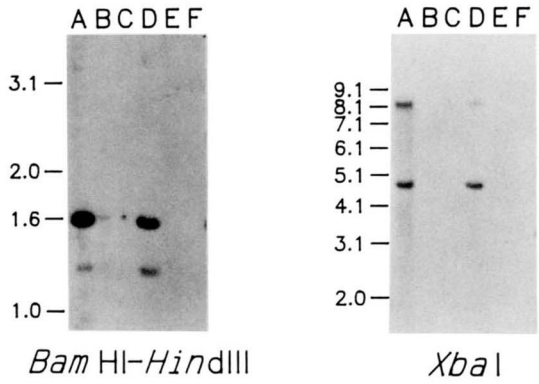

FIGURE 5.—88-10 progeny probed with *Ac*. Southern blots were prepared from six progeny from the 88-10 family following digestion with either *Bam*HI-*Hin*dIII or *Xba*I. Each blot was probed with the 4.3-kb *Ac* probe. Plants A and D have identical *Ac* insertion bands for each digest indicating a germinal transposition occurred in the parent of this family.

TABLE 2

Segregation of lab phenotype in R_2

Progeny	Segregation	
	lab	wt
88-14 A	0	59
B	0	61
C	ND	ND
D	4	11
E	ND	ND
F	ND	ND
G	0	61
H	23	46
I	30	71
J	9	41
K	0	62
L	18	50

ND = not determined.

of *Ac* since this plant was the primary transformant. One progeny, 88-14 F, had these same bands when probed with the appropriate *Ac* probes. This indicated that progeny plant F had inherited the transposed *Ac* which was detected as a somatic band in the parent.

One plant from family 88-14, plant I, had a single new *Ac* insertion as determined by using each of the two *Ac* probes (Figure 6). Unlike the other progeny, there were no resident pMAC fragments of 2.4 kb and 3.6 kb in this plant. Additionally, there was no evidence of the donor plasmid in this progeny when the blot was probed with either *wx*- or T-DNA-specific probes (not shown). As previously discussed, an *Ac* which meiotically segregated from the resident plasmid is an additional indicator of sexual transmission of a transposed element. The insertion site fragments of this germinally transposed *Ac* (5 kb and 6 kb) were also present in progeny D, G and L. These four progeny (D, G, I and L) also had similar *Ac*-specific bands following digestion with *Bam*HI-*Hin*dIII (not shown), further confirming that these bands were

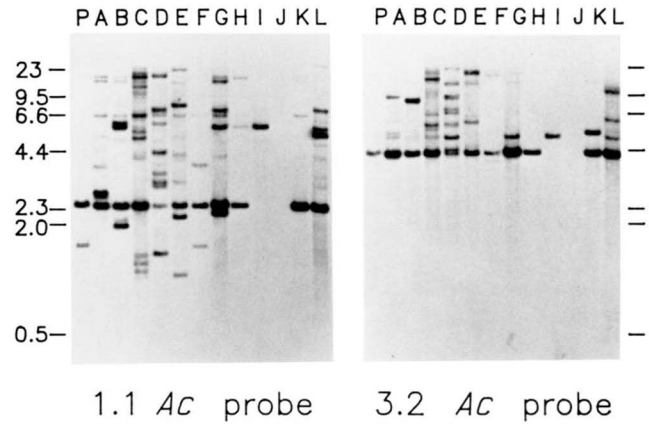

FIGURE 6.—*Xba*I digest of 88-14 progeny. DNA from twelve progeny (A through L) and the parental plant (P) was digested with *Xba*I and Southern blots prepared. The blot was probed with first the 1.1-kb *Ac* probe and later with the 3.2-kb probe. Resident pMAC bands of 2.4 kb and 3.6 kb are seen in many of the plants with each respective probe. Germinally transposed *Ac*s are segregating in progeny D, F, G, I and L (see text).

inherited from the parent. Since these bands are different from those caused by the insertion of *Ac* in 88-14 F, at least two different forward transposition events were sexually transmitted in this family.

While there were at least two forward transposition events segregating in this population, there was no insertion that cosegregated with the lab phenotype. In particular, we did not detect similar insertion bands in the heterozygotes D, H, I, J and L. Progeny J, while segregating for the mutation, had no *Ac* sequences at all. We conclude that the mutation in this progeny population is not marked by a new *Ac* insertion.

Transmission of transposed *Ac* elements at a number of different locations: We similarly analyzed 17 progeny from family 88-01, a population which was segregating for a round leaf shape. In Figure 7, Southern blots of *Xba*I-digested DNA of 15 of the 17 progeny are shown. Progeny 88-01 A–J had the mutant phenotype, K–Q were normal. In this family, a very different pattern was observed compared to the previous families. In 17 progeny examined, none shared the same *Ac* insertion as determined from the banding patterns on two enzyme blots. While some progeny appear to have bands in common when probed with the 3.2-kb *Ac* probe, this similarity does not hold up when the blot is probed with the 1.1-kb *Ac* probe. As such, it was impossible to correlate the mutant phenotype with a particular banding pattern.

There were no resident bands indicative of an untransposed *Ac* detected in either the parent or the progeny using either *Ac* or *wx* sequences as probes; instead, a *wx*-specific empty donor site fragment was observed in 13 of 17 progeny (Figure 7). This suggests an excision event occurred in the parent which was then transmitted to the progeny. Because each progeny has *Ac* at a different location, it is difficult to

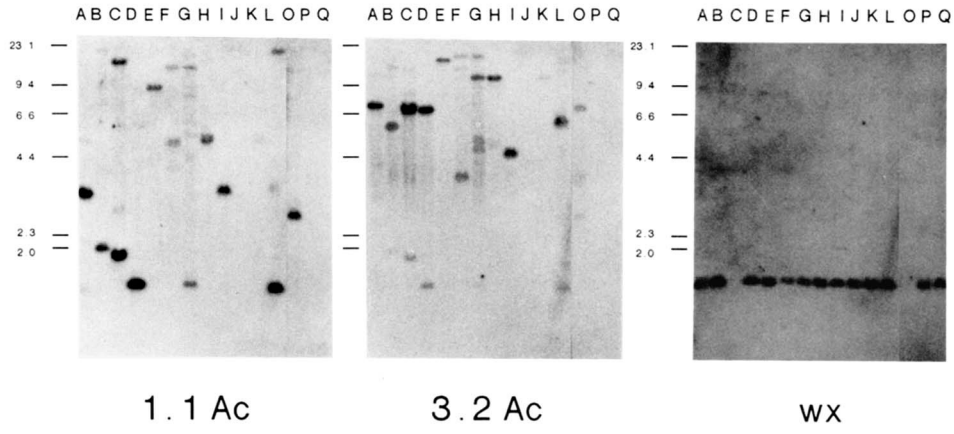

FIGURE 7.—Progeny 88-01 digested with *Xba*I. A Southern blot prepared from an *Xba*I digest of 12 88-01 progeny was probed sequentially with the 1.1 *Ac* probe, the 3.2 *Ac* probe, and the *wx*-specific probe. None of the progeny had transposed *Ac*s at the same location. Progeny C is an example of a plant containing *Ac* but not *wx* sequences.

determine how many different *Ac* insertions were transmitted.

Meiotic assortment of *Ac* and donor pMAC vector: If in germ line cells of the primary transformant *Ac* transposes to a chromosomal position not tightly linked to its resident insertion site, recombination between the transposed *Ac* and donor pMAC could occur. This would be manifested in Southern blots as occasional progeny which contained *Ac* but no donor pMAC sequences. Because we probed our blots with both *Ac* and T-DNA sequences, we could look for recombination between the original *Ac* location and its new insertion site in these families.

Data obtained by probing Southern blots of the 10 families with *Ac*, *wx* and T-DNA border probes is summarized in Table 1. This table indicates the number of progeny with both *Ac* and T-DNA sequences, the number with either *Ac* or T-DNA sequences, and the number with neither. In five out of ten families, progeny were identified that contained *Ac* but not donor plasmid sequences. This means that in at least one half the families, some progeny inherited an *Ac* that transposed a sufficient genetic distance to allow detection of recombination. In total, 6 of 90 progeny had *Ac* but no T-DNA. This is an underestimate of the number of progeny in which *Ac* and T-DNA meiotically assort because even when the sequences are totally unlinked, 9/16 of the progeny will still contain both. Due to the small population sizes, we were not able to estimate map distances of transposition.

Continual transposition of the same element for three generations: Progeny plants which contain a single transposed copy of *Ac* which has meiotically segregated from the T-DNA are valuable for following subsequent behavior of *Ac*. Progeny plant 88-01 O is such a candidate. We sowed self seed from this plant and isolated DNA from seven progeny. The DNA was digested with *Hin*dIII and the resultant Southern hybridization probed with the entire *Ac* sequence found on pJAC-D. Digestion with *Hin*dIII of the transposed element in 88-01 O results in one

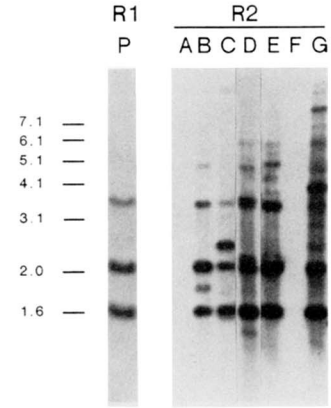

FIGURE 8.—Continual transposition of the same *Ac* into the R_2 generation. DNA from plant 88-01 O (labeled P for parent) and seven self progeny (A through G) was digested with *Hin*dIII and probed with the 4.3-kb *Ac* probe. The progeny exhibit the three bands characteristic of the unique *Ac* insertion present in 88-01 O (1.6-kb internal fragment, 2.2-kb and 3.7-kb border fragments). In addition, however, new bands are present in the progeny indicating somatic transposition in these R_2 plants.

internal fragment of 1.6 kb and junction fragments of 2.2 kb and 3.7 kb (Figure 8). Due to segregation, two of the seven progeny (A and F) did not inherit an *Ac*. The five progeny that harbor *Ac* (B, C, D, E and G) show the same three bands that were present in the parent. However, in addition to these parental bands, new *Ac* insertion sites are apparent. The varying intensities of these bands suggests strongly that they result from somatic transposition of *Ac* in the R_2. Progeny of two other R_1 plants which contained a single copy of *Ac* and no T-DNA (88-01 C and 88-14 I) also exhibited somatic transposition of *Ac* in the R_2 generation (data not shown). We therefore conclude that *Ac* continues to transpose at least up to the third generation following regeneration.

***Ac* can be lost in the R_0:** Family 88-08 contained plants with a high degree of green-yellow sectoring in the leaves. We were interested in examining this mutant because of the similarity of phenotype with other transposon-induced events. DNA was isolated from the parent and 12 mutant progeny, digested with *Bam*HI and *Hin*dIII and probed with *Ac*. None of

these plants contained *Ac* sequences. When the same blots were probed with *wx* sequences, nine of the 12 progeny had a band of the size predicted for an empty donor site. T-DNA border analysis indicated that at least two copies of pMAC had originally integrated into the transformant. Therefore, an event causing the loss of at least two copies of *Ac* occurred early in the development of the parent such that empty donor sites, but not *Ac* insertions, were transmitted to progeny.

DISCUSSION

We examined the segregation of *Ac* elements in progeny of primary transformed tomato plants. Of the ten progeny families examined, four were segregating for aberrant phenotypes. By examining both mutant and wild-type siblings from a given family, we could not detect a correlation between a particular *Ac* insertion and any of the mutations. Indeed, in each family it was possible to identify at least one progeny which exhibited the variant phenotype but did not contain any *Ac* sequences. This analysis determined that a transposed *Ac* was not consistently cosegregating with a mutant phenotype. It is clear from a number of studies that regenerated plants segregate for mutations at a high frequency (EVANS 1988); the mutations we examined most likely result from such somaclonal variation. However, since transposon excision frequently does not regenerate the initial sequence of the insertion site (SCHWARZ-SOMMER *et al.* 1985), the possibility remains that at least some of the mutations may have arisen from these footprints. The high level of *Ac* activity in 88-01 is consistent with this hypothesis.

In order to get a generalized description of the segregation of *Ac* in progeny of primary transformants, we examined six families which were phenotypically wild type in addition to the four aberrant families. We distinguished *Ac* insertions which were genetically transmitted from those that occurred somatically in the R_1 by three criteria: the same insertion was detected in both parental and progeny plants; the same insertion comigrated in at least two siblings; or a meiotic recombination event was detected that resulted in progeny containing a transposed *Ac* element but no T-DNA. These criteria allowed us to arrive at a conservative estimate of the frequency with which transposed *Ac* elements are transmitted from the primary transformant to progeny. This is a conservative estimate because the small sample size imposed by Southern hybridizations reduces the odds of identifying progeny with comigrating insertions and of identifying plants in which *Ac* and T-DNA are assorting.

Even with these limitations, we determined that transposed *Acs* were frequently transmitted to R_1 progeny. By applying the criteria above, we have shown that six of the ten primary transformants transmitted a transposed *Ac* to at least one progeny. Expressed at the progeny level, nearly 30% (26/90) of the progeny inherited a transposed *Ac* from their parent. This proportion of progeny inheriting transposed *Acs* from their parent seems higher than what is observed in maize. A difference in the timing of transposition could lead to such differences if, for example, transposition occurred much earlier in tomato compared to maize. This would lead to a great number of progeny with transposed *Acs*, many of them sharing the same event. Also, mechanisms that attenuate transposition, such as DNA methylation (SCHWARTZ and DENNIS 1986; CHOMET, WESSLER and DELLAPORTA 1987), may not be functional to the same degree in maize and tomato. Thirdly, high germinal transposition rates in primary transformants may result from the transformation and regeneration regime. It will be necessary to measure similar transposition rates in subsequent generations to quantify this observation and determine whether the rates in primary transformants are abnormally high.

We have followed a single *Ac* element through three generations and shown that it remains active at least up to the R_2 generation. This continued activity of *Ac* may prove very valuable in transposon tagging experiments. A given *Ac*-carrying line may be used over time as a continuous source of mutations instead of having to constantly produce more transformants. Also, screening in later generations may prove more efficient due to the absence of somaclonal variants. However, continued high levels of activity may hinder the actual tagging of genes if the element does not remain long enough in the mutated gene. In order to stabilize the mutagenic element in later generations, we are developing a two-element transposon system in tomato (LASSNER, PALYS and YODER 1989).

Ac transposition to a site not tightly linked to the donor T-DNA resulted in meiotic segregation of *Ac* and pMAC donor sequences in R_1 progeny. The populations we examined were not of sufficient size to determine the average genetic distance of germinal transposition. However, since five of ten families showed some assortment of *Ac* from the donor plasmid despite small progeny sizes, it seems likely that transposition to loci loosely linked or unlinked to the T-DNA is not a rare event in tomato.

We have shown that *Ac* can be lost in the primary transformant. Two mechanisms could account for this loss. If *Ac* transposes from one sister chromatid to another at mitosis, a sector could arise consisting of cells without *Ac*. If DNA was prepared from this tissue, apparent loss of the *Ac* element would be observed. This hypothesis, originally put forward by GREENBLATT and BRINK (1963) as an explanation of twinned sectors generated by the maize *Pvv* allele, has since

been confirmed at both the genetic and molecular levels (GREENBLATT 1984; CHEN, GREENBLATT and DELLAPORTA 1987). A second hypothesis is the failure of an excised element to reinsert. One of these events apparently occurred in the parent of 88-08 progeny since an empty donor site but not *Ac* sequences were transmitted to the progeny. From these experiments as well as previous results (YODER *et al.* 1988), we estimate that *Ac* is lost in about 10% of the primary transformants.

Finally, we determined that about half the transformants contained a single copy of the donor plasmid at transformation. This is consistent with results from WALLROTH *et al.* (1986) and JORGENSEN, SNYDER and JONES (1987). From our observations, *Ac* was equally active in plants which were transformed with either one or more *Acs*. Unlike in maize (MCCLINTOCK 1951), we detected no obvious copy number suppression of *Ac* activity when more than one copy of *Ac* was present.

We wish to acknowledge the excellent technical assistance of JOE PALYS. This work is supported by a U.S. Department of Agriculture Competitive Research Grant 86-CRCR-1-1991. F. B. supported by Natural Sciences and Engineering Research Council of Canada.

LITERATURE CITED

BAKER, B., J. SCHELL, H. LOERZ and N. FEDOROFF, 1986 Transposition of the maize controlling element *Activator* in tobacco. Proc. Nat. Acad. Sci. USA **83:** 4844–4848.

BAKER, B., G. COUPLAND, N. FEDOROFF, P. STARLINGER and J. SCHELL, 1987 Phenotypic assay for excision of the maize controlling element *Ac* in tobacco. EMBO J. **6:** 1547–1554.

BERNATZKY, R., and S. D. TANKSLEY, 1986 Genetics of actin-related sequences in tomato. Theor. Appl. Genet. **72:** 314–321.

CHALEFF, R. S., 1981 Application of cell cultures, pp. 93–95 in *Genetics of Higher Plants*, edited by D. R. NEWTH and J. G. TORREY. Cambridge University Press, Cambridge.

CHEN, J., M. I. GREENBLATT and S. L. DELLAPORTA, 1987 Transposition of *Ac* from the *P* locus of maize into unreplicated chromosomal sites. Genetics **117:** 109–116.

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

EVANS, D. A., 1988 Applications of somaclonal variation, in *Biotechnology in Agriculture*, edited by A. MIZRAHI. Alan R. Liss, New York.

FEINBERG, A. P., and B. VOGELSTEIN, 1983 A technique for radiolabelling DNA restriction fragments to a high specific activity. Anal. Biochem. **132:** 6–13.

FRALEY, R. T., S. G. ROGERS, R. B. HORSCH, D. A. EICHHOLTZ, J. S. FLICK, C. L. FINK, N. L. HOFFMAN and P. R. SANDERS, 1985 The SEV system: a new disarmed Ti plasmid vector for plant transformation. Bio/technology **3:** 629–635.

GEISER, M., E. WECK, H.-P. DOERING, W. WERR, U. COURAGE-TEBBE, E. TILLMAN and P. STARLINGER, 1982 Genomic clones of a wild type allele and a transposable element induced mutant allele of the sucrose synthase gene of *Zea mays* L. EMBO J. **1:** 1455–1460.

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.

GREENBLATT, I. M., and R. A. BRINK, 1963 Transpositions of *Modulator* in maize into divided and undivided chromosome segments. Nature **197:** 412–413.

JORGENSEN, R., C. SNYDER and J. JONES, 1987 T-DNA is organized predominantly in inverted repeat structures in plants transformed with *Agrobacterium tumefaciens* C58 derivatives. Mol. Gen. Genet. **207:** 471–477.

KNAPP, S., G. COUPLAND, H. UHRIG, P. STARLINGER and F. SALAMINI, 1988 Transposition of the maize transposable element *Ac* in *Solanum tuberosum*. Mol. Gen. Gen. **213:** 285–290.

LASSNER, M. W., J. M. PALYS and J. I. YODER, 1989 Genetic transactivation of *Dissociation* elements in transgenic tomato plants. Mol. Gen. Gen. (in press).

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

MCCORMICK, S., J. NIEDERMEYER, J. FRY, A. BARNASON, R. HORSCH and R. FRALEY, 1986 Leaf disk transformation of cultivated tomato (*L. esculentum*) using *Agrobacterium tumefaciens*. Plant Cell Rep. **5:** 81–84.

RICK, C. M., AND L. BUTLER, 1956 Cytogenetics of the tomato. Adv. Genet. **8:** 267–382.

RICK, C. M., and J. I. YODER, 1988 Classical and molecular genetics of tomato: highlights and perspectives. Annu. Rev. Genet. **22:** 281–300.

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

SCHWARZ-SOMMER, Z., A. GIERL, H. CUYPERS, P. A. PETERSON and H. SAEDLER, 1985 Plant transposable elements generate the DNA sequence diversity needed in evolution. EMBO J. **4:** 591–597.

UPADHYAYA, K. C., H. SOMMER, E. KREBBERS and H. SAEDLER, 1985 The paramutagenic line *niv*-44 has a 5 kb insert, Tam2, in the chalcone synthase gene of *Antirrhinum majus*. Mol. Gen. Genet. **199:** 201–207.

VAN SLUYS, M. A., J. TEMPE and N. FEDOROFF, 1987 Studies on the introduction and mobility of the maize *Activator* element in *Arabidopsis thaliana* and *Daucus carota*. EMBO J. **6:** 3881–3889.

WALLROTH, M., A. G. M. GERATS, S. G. ROGERS, R. T. FRALEY and R. B. HORSCH, 1986 Chromosomal localization of foreign genes in *Petunia hybrida*. Mol. Gen. Genet. **202:** 6–15.

YODER, J. I., J. PALYS, K. ALPERT and M. LASSNER, 1988 *Ac* transposition in transgenic tomato plants. Mol. Gen. Genet. **213:** 291–296.

Communicating editor: S. D. TANKSLEY