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Genetics

Transposition of the maize controlling element "Activator" in tobacco

(plant transformation/transposon tagging/Ti plasmid)

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ABSTRACT **Transposition of the maize autonomous controlling element Activator (*Ac*) and a nonautonomous derivative, Dissociation (*Ds*), was investigated in tobacco cells. Tobacco protoplasts were transformed with Ti-plasmid vectors that contained *Ac* or *Ds* flanked by short maize *wx* gene sequences. The structures of the elements and surrounding *wx* and T-DNA sequences were investigated in nine *Ac* and five *Ds* tobacco transformants by digestion with restriction enzymes, Southern blotting, and hybridization using specific probes. In four of the nine *Ac* transformed lines, *Ac* had excised from its original position in the T-DNA and inserted at new sites in the tobacco genome. *Ds* did not excise from its original T-DNA position in any of the transformants examined. Two *Ac* fragments and cellular flanking sequences were cloned from a line of tobacco in which *Ac* had transposed. Fragments, comprised of sequences flanking the newly integrated *Ac* elements, were used as hybridization probes to normal tobacco DNA and to the tobacco DNA from which they were isolated. The *Ac* copies were integrated into repetitive tobacco DNA sequences. Two tobacco fragments containing empty *Wx* donor sites were cloned from the DNA of the same *Ac* transformant and sequenced. Both sequences are among the types of excision products observed to result from *Ac*-catalyzed excision events in maize. Our results indicate that the maize controlling element *Ac* is capable of self-catalyzed transposition in tobacco.**

The maize transposable controlling element Activator (*Ac*) was first identified and studied genetically by Barbara McClintock (ref. 1; for review see ref. 2). The *Ac* element is capable of transposing autonomously, and it can also *trans*-activate the transposition of a group of elements collectively designated Dissociation (*Ds*) elements. *Ac* and *Ds* elements comprise a maize transposon family. Many elements of this family have been cloned and subjected to structural analysis (3–7). The *Ac* element is a small, 4.5-kilobase (kb) transposon that has an 11-base-pair (bp) terminal inverted repetition and generates an 8-bp duplication upon insertion. Sequence analysis of the element has revealed the presence of three major open reading frames (ORFs), two of which overlap (Fig. 1; refs. 8 and 9).

Some insight into element-encoded functions has been gained from the study of *Ds* elements. Genetically, *Ds* elements are defined by their ability to transpose only in the presence of an *Ac* element. The results of molecular analyses have shown that *Ds* elements comprise a structurally heterogeneous group of elements, all of which have similar or identical 11-bp terminal inverted repetitions, but only some of which are closely related to *Ac* in structure. Several *Ds* elements that arose directly from an *Ac* element by spontaneous mutations have been analyzed and found to have sustained internal deletions, the smallest of which affect only ORF 1 or overlap ORF 1 and ORF 2 (4, 8, 10). Since a *Ds* element cannot promote its own transposition but can be activated to transpose by an *Ac* element, the deletions must affect the structure or expression of the sequence encoding the element's transposition function.

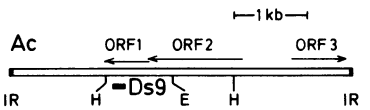

FIG. 1. A diagrammatic representation of the *Ac* element. The element is 4.5 kb in length and has 11-bp terminal repetitions (IR) that are indicated by the dark lines at the ends of the element. Arrows indicate the length and polarity of the three largest ORFs of the elements (8). The bar below *Ac* indicates the sequence that is deleted in the *Ds*9 element (4, 8). *Hin*dIII (H) and *Eco*RI (E) restriction enzyme sites are indicated.

To facilitate the further molecular genetic analysis of *Ac*-encoded gene products, as well as to explore the possibility of using *Ac* as a mutagen and gene tag in plants other than maize, we introduced a cloned *Ac* element into tobacco cells on an *Agrobacterium tumefaciens* Ti plasmid. Here we present evidence that *Ac* catalyzes its own transposition at a high frequency in tobacco cells.

MATERIALS AND METHODS

Bacterial Strains. *A. tumefaciens* strain C58C1*rif* (11) containing the pGV3850 Ti plasmid was recombined with *Escherichia coli* plasmids pBL1103-Ac, pBL1103-Ds, and pBL1103-Wx according to Van Haute (12). The resulting *A. tumefaciens* strains pGV3850::pBL1103-Ac, pGV3850::pBL1103-Ds, and pGV3850::pBL1103-Wx were designated pTi-Ac, pTi-Ds, and pTi-Wx, respectively, and used to transform tobacco protoplasts.

Construction of pBL1103-Ac, pBL1103-Ds, and pBL1103-Wx *E. coli* Plasmids. The 247-bp *Pst* I *Wx* maize revertant fragment from *Wx 9-rl* (4) was inserted into pUC8 at the *Pst* I site. The *Wx* fragment was then removed from the vector by *Hin*dIII and *Hin*cII double digestion. The *Wx* fragment was blunt-ended and inserted into *Bcl* I-digested and filled in pLGV1103neo vector (13). The resulting *Wx* clone was designated pBL1103-Wx. The plasmids, pBL1103-Ac and pBL1103-Ds, were constructed by substituting either the *Ac* or *Ds Pst* I fragments (4) for the *Wx Pst* I fragment of pBL1103-Wx.

Transformation of Tobacco Protoplasts and Screening of Transformants. A modified cocultivation system (13, 14) was used to transform isolated *Nicotiana tabacum* cv. Petit Havana SR1 tobacco leaf protoplasts. Transformants were screened for the presence of nopaline. Colonies approximately 2 mm in diameter were transferred from 1-month-old

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Abbreviations: kb, kilobase(s); bp, base pair(s); ORF, open reading frame.

cocultivation bead culture to MS medium (naphthleneacetic acid at 1 mg/liter, kinetin at 0.2 mg/liter). When colonies grew to 5–7 mm in diameter they were divided in half. One-half of the callus was placed on fresh MS medium, and the other half of the callus was tested for the presence of nopaline (15). Approximately 10% of the callus colonies tested after transformation were nopaline-positive. Nopaline-positive callus was grown further on MS medium and repeatedly checked for the presence of nopaline. A portion of callus tissue was later placed on MS medium containing 6-benzylaminopurine at 0.5 mg/liter, naphthleneacetic acid at 0.1 mg/liter to induce the formation of shoots.

Preparation and Analysis of DNA Isolated from Tobacco Tissue. Plant DNA was prepared as described (13, 16). DNA was digested with restriction enzymes, separated by electrophoresis on 1% agarose gels, transferred to nitrocellulose, and hybridized. Radioactively labeled DNA probes were produced by nick-translation to a specific activity of 1×10^8 cpm/μg. Purified DNA fragments used to generate the probes were the 4.8-kb *Pst* I *Ac*9 fragment (Ac probe) and the 247-bp *Wx* revertant fragment (Wx probe). (Full documentation of restriction enzyme mapping data will be provided upon request.)

Cloning of Genomic Fragments. Tobacco genomic DNA was isolated from an established teratoma-like shoot culture of pTi-Ac transformant line 30 and partially digested with *Sau*3A and ligated into the *Bam*HI cloning site of λEMBL 4 (17). The resulting recombinant phage were screened by plaque hybridization with Ac and Wx probes. Two positive λ *Ac* clones were further subcloned. *Eco*RI fragments that consisted in part of *Ac* sequences and in part of tobacco genomic DNA were subcloned into pUC8 and designated TAc2 and TAc3. The inserted *Eco*RI 2.9-kb fragment of TAc2 was isolated and used as a probe. The 5.6-kb *Eco*RI-inserted fragment of TAc3 was isolated from vector DNA. A subfragment that consisted of approximately 100 bp of one of the ends of the *Ac* element and approximately 3.1 kb of flanking tobacco genomic DNA was isolated and used as a probe to identify cellular integration sites of *Ac*.

DNA from two λEMBL 4 clones that hybridized to the Wx probe was isolated to study the nucleotide sequence of the empty donor site. The *Pst* I fragments with homology to the Wx probe were subcloned into the *Pst* I site of M13mp8 phage DNA (18), and the nucleotide sequence of the insert was determined (see Fig. 4).

RESULTS

Construction of Plasmids Containing Maize Transposons. To assess the ability of *Ac* to transpose in tobacco, we transferred the following three maize sequences into tobacco cells: an *Ac* element inserted into a fragment of the maize "Waxy" (*wx*) locus, a *Ds* element on a comparable *wx* locus fragment, and a fragment of the *wx* locus representing an empty donor site. The empty donor site is the corresponding fragment of the *wx* locus from which the transposable element excised in the *Wx* revertant allele designated *Wx9-rl* (4, 8). The maize DNA fragments used in these constructions have been described in detail (4), and their important properties are reviewed here. In the *Acwx-m9* maize allele from which the *Ac* element was originally cloned, the 4.56-kb *Ac* element was inserted into a 241-bp *Pst* I fragment of the *wx* locus, generating a 4.8-kb *Pst* I fragment with no internal *Pst* I sites. Subsequent to the insertion of the *Ac* element in the *wx* locus, the element sustained a spontaneous internal deletion that converted the *Ac* element to a transposition-defective *Ds* element (4). The *Ds* element was cloned from the derivative strain, designated *wx-m9*, and was found to have a 0.2-kb deletion in ORF 1 of *Ac* (Figs. 1 and 2). The empty donor site is a *Pst* I fragment cloned from a revertant *Wx* strain in which the *Ac* element had excised from its original insertion site. This *Pst* I fragment, designated the *Wx* fragment, is 247 bp long because 6 bp of the 8-bp duplication generated upon insertion of the *Ac* element were retained upon its excision in the maize plant (8).

The three *Pst* I fragments described above were inserted at the *Bcl* I site of pLGV1103neo (13). The derivative plasmids containing the *Ac*, *Ds*, and *Wx* maize sequences have been designated pBL1103-Ac, pBL1103-Ds, and pBL1103-Wx, respectively. The plasmids, which were constructed in *E. coli*, were transferred to *A. tumefaciens* strain C58C1 containing the Ti plasmid pGV3850. Cointegrates of the *E. coli* plasmids and pGV3850 were recovered (11, 12). To verify the structure of the cointegrates, DNA was extracted from the selected *A. tumefaciens* strains and subjected to restriction endonuclease mapping. The cointegrates, designated pGV3850::pBL1103-Ac, pGV3850::pBL1103-Ds, and pGV3850::pBL1103-Wx (and subsequently referred to here as pTi-Ac, pTi-Ds, and pTi-Wx, respectively for simplicity) had the expected T-DNA structure, shown in Fig. 2. No unanticipated restriction fragments were observed in *A. tumefaciens*, indicating that the maize transposable elements are stable in bacteria. Since the structure shown in Fig. 2 is bounded by T-DNA border sequences, we anticipated the transfer of the entire fragment into plant cells (19).

Transformation of Tobacco Cells with pTi-Ac, pTi-Ds, and pTi-Wx. Protoplasts isolated from SR1 tobacco plantlets were transformed by cocultivation with *A. tumefaciens* strain C58C1 containing the pTi-Ac, pTi-Ds, and pTi-Wx plasmids. Transformed callus was grown for extraction and analysis of the genomic DNA.

***Ac* Transposes in Tobacco Cells.** DNAs from nine cell lines independently transformed with pTi-Ac have been examined, and four have yielded evidence of *Ac* transposition. DNA was extracted from transformed callus tissue, digested with restriction enzymes, and probed with *Ac* and *Wx* sequences to investigate the structure of the transformed T-DNA. Examples of the two different types of results obtained with transformed callus containing *Ac* are shown in Fig. 3. Fig. 3 *a*, lanes 3 and 4, and *b*, lanes 3 and 4, exemplifies the results obtained with callus that gave no evidence of *Ac* movement. Ac and Wx probes detect fragments of the size originally introduced. Thus, *Pst* I digests contain only the input maize 4.8-kb fragment comprising the *Ac* element and its flanking *wx* sequence. *Pst* I/*Hin*dIII digests likewise contain only those sequences expected from cleavage of the element at the two internal *Hin*dIII sites, as well as the flanking *Pst* I sites. These fragments are an internal 1.6-kb *Ac* fragment and *Ac-wx* border fragments of 1.3 and 1.9 kb with homology to both the Ac and Wx probes (the double digest also contains a small amount of a 4.8-kb fragment homologous to the Wx probe, probably as a result of incomplete *Hin*dIII digestion). No small fragments with homology to the 247-bp Wx probe are observed. Analysis of the other plasmid sequences originally located within the T-DNA borders of the donor pTi-Ac plasmid indicated that the structure of the DNA segment integrated in the genome was that diagrammed in Fig. 2 (data not shown). Although the number of T-DNA copies present in the transformed lines has not been assessed accurately, it appears to be between one and three copies in the various lines analyzed (16, 19).

By contrast to the five cell lines in which the *Ac* elements are stable, four cell lines showed evidence of *Ac* transposition. An example of the results obtained with one such cell line is given in lanes 1 and 2 of Fig. 3 *a* and *b*. DNA of this cell line yielded many *Pst* I fragments with homology to the *Ac* elements. These ranged in size from smaller than the original 4.8-kb *Ac*-containing *Pst* I fragment to much larger. When the same DNA was probed with the maize *Wx*

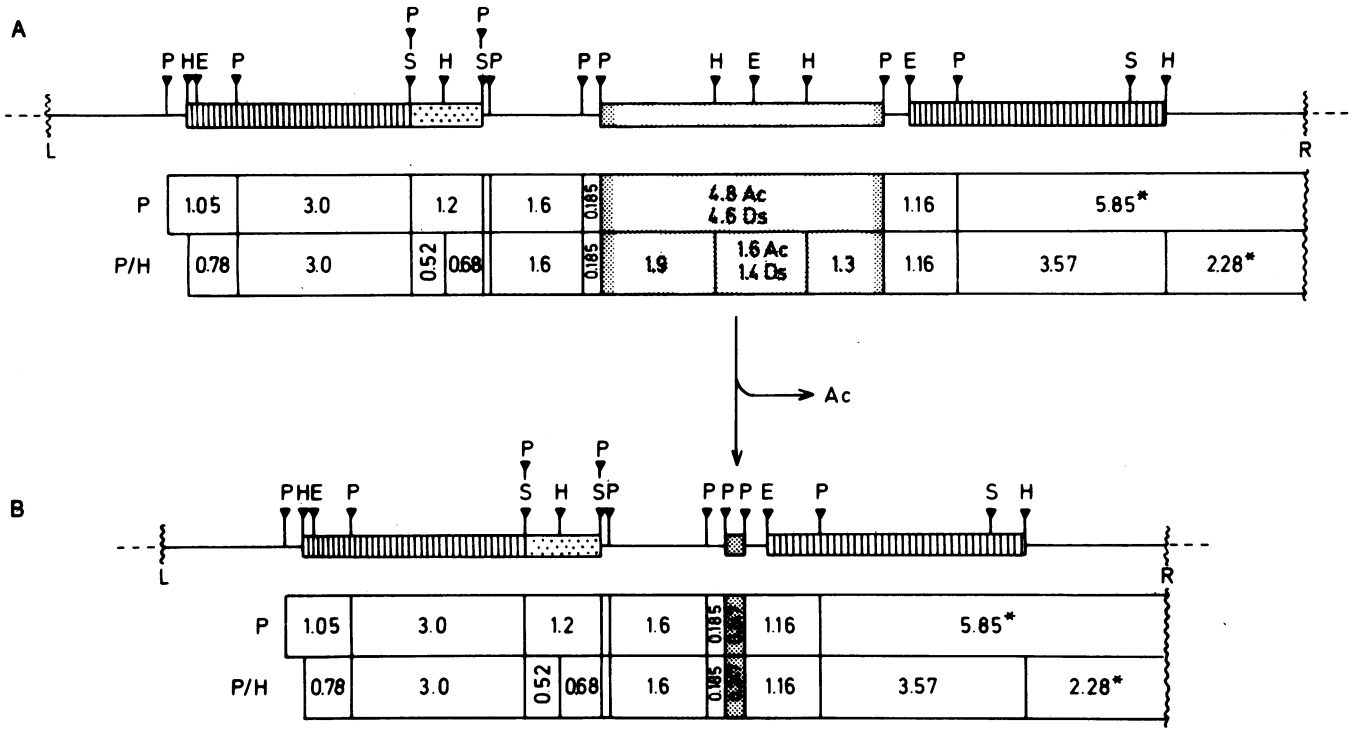

FIG. 2. Structures of T-DNA of cointegrate Ti plasmids of *A. tumefaciens* strains pTi-Ac, pTi-Ds, and pTi-Wx. Total *A. tumefaciens* DNA was isolated from each strain, and the structure of the T-DNA was determined by Southern blot analysis. *Pst* I (P), *Hin*dIII (H), *Eco*RI (E), and *Sal* I (S) restriction enzymes were used. (*A*) pTi-Ac and pTi-Ds T-DNA structures. pTi-Ac contains a 4.8-kb *Pst* I fragment consisting of the 4.5-kb *Ac* element flanked by 121 bp and 128 bp of *wx* gene sequence on the right and the left, respectively. pTi-Ds contains a 4.6-kb *Pst* I fragment consisting of the 4.3-kb *Ds* element flanked by the same *wx* sequences as in pTi-Ac. (*B*) T-DNA structure of pTi-Wx. pTi-Wx contains a 247-bp *Wx* fragment cloned from a maize *Wx* revertant strain. The *Ac* and *Ds* sequences are indicated by the medium stippling in *A*. The *wx* sequences are indicated by the darker stippling in *A* and *B*. The T-DNA fragments that flank the *Ac*, *Ds*, or *Wx Pst* I fragments are identical in the three strains. The vertically striped regions that flank the *Ac*, *Ds*, and *Wx* fragments correspond to pBR322 sequences. The sparsely stippled region adjacent to the leftward pBR322 region is comprised of Tn*903* sequences. The numbers drawn within the boxed areas are the sizes of relevant *Pst* I or *Pst* I–*Hin*dIII DNA fragments. The 5.85-kb *Pst* I and 2.28-kb *Pst* I–*Hin*dIII right most T-DNA fragments are indicated by an * and correspond to the distance to the rightward border sequence (R) located within the Ti cointegrate plasmid. The orientations of the *Ac* and *Ds* sequences are reversed to the orientations drawn in Fig. 1.

fragment, only the following three fragments were detected: a 4.8-kb, an at least 11-kb, and a 240–250-bp fragment. This observation implies that most of the *Ac* sequences are no longer associated with the original flanking maize *wx* sequence. That the flanking sequences rather than the internal sequences of *Ac* have changed is suggested by the observation that the *Pst* I/*Hin*dIII double digest contains multiple copies of the 1.6-kb internal *Ac* fragment, but few copies of the original *Ac-wx* border fragments of 1.3 and 1.9 kb (Fig. 3*a*, lanes 1 and 2). Longer fragments of several different sizes are detected instead, most of which are not homologous to the Wx probe. The presence of a *Pst* I fragment that is homologous to and comigrates with the 247-bp *Wx* fragment detectable in plants transformed by the pTi-Wx plasmid (Fig. 3*b*; lanes 1, 2, 5, and 6) suggests that excision events not unlike those observed in maize have occurred in the tobacco cells.

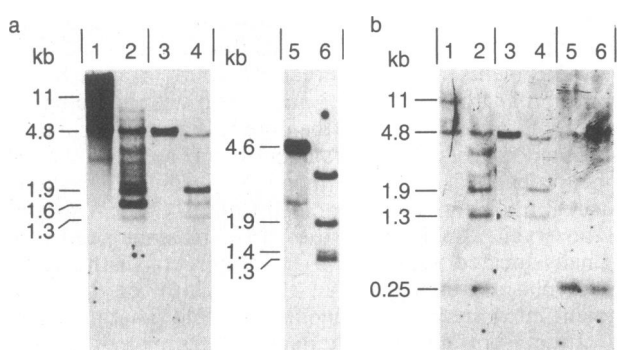

FIG. 3. Genomic DNA isolated from pTi-Ac, pTi-Ds, and pTi-Wx tobacco transformants. Genomic DNA was isolated from individual tobacco pTi-Ac and pTi-Ds transformed callus lines. Ten individually transformed pTi-Wx callus lines were pooled prior to DNA isolation. Genomic DNA (10 μg) of each isolate was digested with *Pst* I (*a* and *b*, lanes 1, 3, and 5) or *Pst* I and *Hin*dIII (*a* and *b*, lanes 2, 4, and 6), electrophoresed on agarose gels, transferred to nitrocellulose filters, and analyzed by hybridization to Ac (*a*) and Wx probes (*b*). pTi-Ac transformed line 30 (*a* and *b*, lanes 1 and 2), pTi-Ac transformed line 34 (*a* and *b*, lanes 3 and 4), pTi-Wx transformants (*b*, lanes 5 and 6), and pTi-Ds transformed line 2 (*a*, lanes 5 and 6).

The *Ds* Element Does Not Transpose. Several cell lines transformed by the pTi-Ds plasmid have been subjected to similar analyses. We have examined five cell lines and have found no evidence of *Ds* transposition. A representative analysis of such a cell line is shown in Fig. 3*a*, lanes 5 and 6, and yields essentially the same results as obtained with the stable *Ac*-containing cell lines (lanes 3 and 4 in Fig. 3 *a* and *b*). These results provide evidence that the transposition observed in *Ac*-containing cell lines is dependent on a gene product of the *Ac* element itself.

The Structure of Empty Donor Sites. Empty donor sites resulting from excisions of *Ac* in tobacco transformants were cloned from DNA isolated from a teratoma-like subline derived from the original callus whose DNA is analyzed in lanes 1 and 2 in Fig. 3 *a* and *b*. The subline contained several empty donor sites, as well as several copies of *Ac* elements. DNA fragments were cloned into the λEMBL4 vector and screened with a plasmid carrying the *Wx* 247-bp maize fragment. Two different tobacco fragments containing a small *Pst* I fragment with homology to the Wx probe were recovered and analyzed. The structure of the fragments was that

expected for an otherwise unrearranged pTi-Ac T-DNA from which the *Ac* element had been excised. That is, both contained fragments with homology to Tn*5* and pLGV1103-neo that comigrated with the fragments of the original plasmid (data not shown). The *Pst* I fragment with homology to the maize *Wx* fragment was subcloned from both and sequenced. As illustrated in Fig. 4, one of the *Wx* fragments (T-wx 1) recovered from the tobacco genome has precisely the same sequence as the maize *Wx* revertant fragment, *Wx9-r1*. That is, the original insertion site is marked by a 6-bp duplication representing part of the 8-bp duplication generated upon insertion of the element. The other fragment, T-wx 2, gives a similar, but not identical, sequence that contains an 8-bp duplication whose central two nucleotides are a thymidine and a guanosine residue rather than the adenosine and cytidine residues of the original duplication. Both are among the types of *Ac* excision products observed in maize (6, 20). It appears, therefore, that *Ac* elements excise in tobacco by a mechanism similar to or identical with that in maize.

Ac Element Insertion Sites in Tobacco DNA. Further evidence that *Ac* elements have transposed from their initial insertion sites within the *wx* sequence of the T-DNA in pTi-Ac T-DNA was obtained by cloning *Ac*-containing fragments from transformed tobacco cells. The recombinant λ library that was used for the isolation of empty *Wx* donor sites was also screened for fragments having homology to the *Ac* element. Two clones containing different portions of the *Ac* element and flanking sequences were identified and analyzed. One clone, T Ac3, contained approximately 50% of the right end (Fig. 1) of the *Ac* sequence, as well as approximately 3 kb of flanking sequence, while a second clone, T Ac2, contained approximately 30% of the element's left end and about 1.5 kb of flanking sequence. Neither clone hybridized to the 247-bp *Wx* maize fragment in which the *Ac* element was originally inserted nor to the pLGV1103neo sequences immediately adjacent to it in the T-DNA segment of the pTi-Ac plasmid (data not shown). Both subclones hybridized to repetitive tobacco DNA sequences (Fig. 5). Although it is not known whether the two clones represent the same insertion site, it appears likely that they do not, because rather different arrays of repetitive tobacco DNA fragments are detected by the two *Ac*-containing subclones, and they do not cross-hybridize (data not shown). Thus the *Ac* elements cloned from the DNA of tobacco cell lines transformed by the pTi-Ac plasmid are no longer integrated in the original plasmid sequence but are inserted into tobacco DNA sequences.

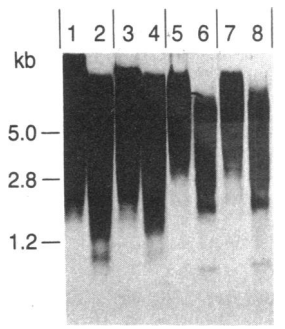

FIG. 5. *Ac* element insertion sites in tobacco DNA. Genomic DNA was isolated from an untransformed tobacco plant (lanes 1, 2, 5, and 6) and from a teratoma-like subline of pTi-Ac transformed line 30 (lanes 3, 4, 7, and 8). The DNA was digested with *Pst* I and *Hin*dIII (lanes 1, 3, 5, and 7) or *Eco*RI and *Bgl* II (lanes 2, 4, 6, and 8); 10 μg of DNA was electrophoresed on identical 1% agarose gels, transferred to nitrocellulose filters, and hybridized to T Ac2 (lanes 1–4) and T Ac3 (lanes 5–8) probes.

DISCUSSION

We have presented several lines of evidence that the maize transposable element *Ac* functions to promote its own transposition in tobacco cells by the same mechanism that operates in maize cells. Tobacco protoplasts were transformed with a Ti plasmid carrying the maize *Ac* element flanked by maize *wx* locus sequences. In almost half of the regenerated tobacco callus cell lines, the maize element had transposed to new sites, leaving behind an empty donor site comparable in structure to those observed in maize.

Sequence analysis of two empty donor sites cloned from tobacco DNA revealed their similarity to those that have been analyzed in maize. It has been observed that plant transposable elements frequently undergo a unique type of imperfect excision. The *Ac* element, which in maize generates an 8-bp duplication on insertion, commonly leaves behind part or all of the duplication, either intact or in a slightly modified form (20). One of the donor sites recovered from the tobacco genome had a 6-bp duplication at the former insertion site and was identical to the sequence observed in a spontaneous maize revertant of the *Ac* insertion into the *wx*

Wild-type	Wx	-CATGGAGA-
Ac insertion	Ac wx-m9	-CATGGAGA---Ac---CATGGAGA-
Wx revertant (maize)	Wx 9-r1	-CATGGAGA..TGGAGA-
Empty wx donor site (tobacco)	T-wx1	-CATGGAGA..TGGAGA-
Empty wx donor site (tobacco)	T-wx2	-CATGGAGTCATGGAGA- (* *)

FIG. 4. Comparison of sequences at the former *Ac* insertion site. The sequence of the maize *Wx* wild-type insertion site is compared with that of maize *Ac* insertion *Ac wx-m9* (8), the maize revertant *Wx9-r1* (8), and two empty donor sites isolated from tobacco. The sequence of the wild-type *Wx* insertion site is given on the top. The *Ac wx-m9* 8-bp insertion site is directly repeated and shown in the second line. The sequence of the corresponding region of the *Wx* revertant allele *Wx9-r1* is shown in the third line. It contains a 6-bp duplication at the original *Ac* insertion site. The sequence of two empty *wx* donor sites of tobacco, T-wx1 and T-wx2, are given in the last two lines, respectively. The T-wx2 sequence contains an 8-bp duplication that differs by 2 bp (*) from the original duplication.

locus, while the second site had a slightly different sequence, retaining an 8-bp duplication the central 2 bp of which differ from that of the original duplication by transversions. Several similar empty donor site sequences with central transversions have been reported in maize (20, 21). Hence the *Ac* excision mechanisms must be similar in tobacco and in maize.

It is also likely that an *Ac* gene product is involved in the excision in tobacco, as it is in maize. Five different tobacco lines transformed by a Ti plasmid containing a transposition-defective *Ds* derivative of the *Ac* element have been examined and show no evidence of transposition. Only those restriction fragments present in the pTi-Ds plasmid were observed in the DNA of the transformed cells. The most likely explanation of this observation is that the promoters of the *Ac* gene product(s) involved in transposition are functional in both monocots and in dicots. An alternative possibility is that the *Ac* genes are expressed by readthrough from external promoters. However, the orientation of ORF 1 and ORF 2 of the *Ac* element is opposite to the direction of transcription from the adjacent pNOS promoter carried by the input pBL1103-Ac plasmid. Thus it appears more likely that the *Ac* element gene product(s) that participates in transposition in tobacco is expressed from *Ac* promoter sequences.

There is evidence that *Ac* transposed to new sites within the tobacco genome. Most of the restriction fragments homologous to *Ac* observed in pTi-Ac transformed lines show no homology to the *wx* sequences within which the *Ac* element was originally inserted in the T-DNA of the pTi-Ac cointegrate. Moreover, fragments of *Ac* and flanking sequences cloned from the tobacco genome of a transformed cell line show no homology to the *wx* and bacterial plasmid sequences within which the *Ac* element was integrated when introduced into the tobacco cells.

The detection of empty *Wx* donor sites in tobacco, combined with the observation of *Ac* insertion into new sites within the tobacco genome, suggests that *Ac* transposition in tobacco occurs by the same type of nonreplicative mechanism as in maize. If transposition in tobacco occurred by a replicative mechanism resembling that used by the Tn*3* transposon of bacteria (22), and possibly the Mu transposon of maize (23), the appearance of new *Ac* elements would not be expected to coincide with the appearance of empty donor sites. Since all cell lines that show new insertion sites have empty *Wx* donor sites, it follows that *Ac* transposition occurs by a nonreplicative mechanism in tobacco, as it does in maize (2, 21). The increased copy number of *Ac* in transformants of the type illustrated in Fig. 3*a*, lanes 1 and 2, suggests that additional copies of the element have accumulated. This is not unexpected, since it has been observed in maize that elements frequently insert into an unreplicated chromosomal site (24).

Although we have no precise means of comparing the frequency of *Ac* transposition in tobacco and in maize, we have been quite surprised by the high frequency of *Ac* transposition detected in the present experiments. Almost half of the *Ac*-containing tobacco cell lines examined gave evidence of *Ac* transposition. In some of the cell lines, most or all of the several *Ac* T-DNA copies had lost their inserted *Ac* elements and had empty donor *Wx* sites. Evidence has begun to accumulate that transposable elements, including the *Ac* element, can be inactivated by modifications of element sequences (25). It is conceivable that mechanisms that regulate the *Ac* transposition frequency in maize may either not operate in tobacco or may not take effect for some time after the introduction of the element. It is known that the *Ac* element transposition frequency in maize decreases and is developmentally delayed with increasing numbers of *Ac* elements in maize (2, 21). It may also be that a newly introduced element can undergo frequent transpositions prior to the accumulation of regulatory proteins.

The ability of *Ac* to transpose in dicots opens several interesting possibilities for further experimentation. *Ac* element functions can be analyzed in substantial detail by *in vitro* mutagenesis, using *Agrobacterium* vectors to introduce mutated *Ac* elements into cells readily transformed by *Agrobacteria*. Indeed, the design of the present vector and others like it in which the transposable element is inserted in such a way that it disrupts expression of a selectable or visible marker should permit the accurate determination of excision frequencies promoted by normal, mutated, and genetically engineered transposable elements. The *Ac* element could also prove useful in mutating and marking genes for cloning. The *Ac* element has already been used for gene isolation in maize (2, 26), and its utility in other plants is likely to depend only on the relative ease with which *Ac* insertions can be correlated with mutant phenotypes, since sequences homologous to *Ac* are unlikely to be present in distantly related plants. In the course of the present experiments, for example, we have found no evidence that tobacco plants contain sequences that cross-hybridize significantly with *Ac* under stringent conditions. Thus any new mutant allele that can be attributed to insertion of the *Ac* element can readily be used to clone the corresponding gene.

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