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Variable Patterns of Transposition of the Maize Element *Activator* in Tobacco

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The strategy to be followed in a transposon tagging experiment will be determined largely by the transposition pattern of the transposon in question. With a view to utilizing the maize element *Activator* (*Ac*) as a transposon tag in heterologous systems, we investigated the pattern of *Ac* transposition from six different loci in transgenic tobacco. We isolated germinal revertants from plants carrying mutable alleles of the antibiotic-resistant gene streptomycin phosphotransferase (SPT) and mapped the location of the transposed *Ac* (*trAc*) elements relative to the donor SPT gene. A comparison of the distributions of *trAc*s among the six loci revealed that, although the receptor sites for *trAc*s tend to be linked to the donor locus, the pattern of *Ac* transposition in tobacco displays surprising locus-to-locus variation. Some *trAc* distributions showed the same tight clustering around the donor locus previously seen in maize, whereas others were more dispersed. The possible meaning of these findings and their implication for transposon tagging in heterologous systems are discussed.

INTRODUCTION

Transposon tagging, i.e., the isolation of genes using transposable elements as probes, has been applied successfully in plants such as maize and *Antirrhinum*, where the genetics of transposable elements has been studied extensively (e.g., Cone et al., 1988; Carpenter and Coen, 1990). This success has triggered efforts to develop genetic systems that would allow the utilization of the maize and snapdragon elements as gene isolation tools in heterologous species lacking well-characterized transposons (Baker et al., 1986, 1987; Jones et al., 1989, 1990a; Martin et al., 1989; Masson and Fedoroff, 1989).

Three autonomous or self-mobilizing transposons have been tested to date: the maize elements *Activator* (*Ac*) and *Suppressor-mutator/Enhancer* (*Spm/En*) and the *Antirrhinum* element *Tam3*. *Tam3* does not appear to be very active in transgenic tobacco: it becomes methylated and largely inactivated by the new host plant (Martin et al., 1989). *Spm/En* transposes in tobacco (Masson and Fedoroff, 1989; Pereira and Saedler, 1989) and potato (Frey et al., 1989), but its activity may be affected by aberrant transcript processing. The most promising element appears to be *Ac*, the first autonomous element described by McClintock (1951). *Ac* has been shown to transpose in a variety of dicots: tobacco (Baker et al., 1986), *Arabidopsis* and carrot (Van Sluys et al., 1987), tomato (Yoder et al., 1988), potato (Knapp et al., 1988), and petunia (Haring et al., 1989), and it does not appear to be methylated in tobacco (Nelsen-Salz and Döring, 1990) or *Arabidopsis* (J. Keller, unpublished observations).

The efficiency of a genetic experiment designed to tag a specific gene with a transposon will depend on the pattern of transposition of the element in question from the starting "donor" locus. Our knowledge of the pattern of transposition of *Ac* in maize derives largely from extensive genetic studies with just two mutable alleles, *P-vv* and *bz-m2*, which harbor, respectively, *Ac* elements at the *P* and *bz* loci (Greenblatt, 1984; Dooner and Belachew, 1989). These studies have led to the view that *Ac* in maize transposes preferentially to closely linked sites: 61% and 53% of all transpositions from *P-vv* and *bz-m2*, respectively, are to linked sites and, of these, the majority are to sites within 5 centimorgans (cM) of the donor locus.

We previously mapped the location of transposed *Ac* (*trAc*) elements relative to one donor locus in transgenic tobacco (Jones et al., 1990a). We found a pronounced preference for transposition to very closely linked sites: 71% of all *trAc*s mapped within 1 cM of the donor locus. If this represented a common transposition pattern in transgenic hosts, the value of *Ac* as a transposon tag in heterologous systems would be limited. To examine whether this pattern was generally true of other loci in tobacco, we mapped the location of *trAc* receptor sites relative to six additional loci. We found that although the receptor sites tend to be linked to the donor locus, the pattern of *Ac* transposition in tobacco displays surprising locus-to-locus variation.

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RESULTS

Assay for *Ac* Excision and Measurement of Transposition Distance

Our reporter gene for *Ac* excision is a bacterial streptomycin phosphotransferase gene (SPT) engineered for expression in plants. In the presence of streptomycin, transgenic seedlings carrying this gene (SPT+) are green, those lacking it (SPT−) are white, and those carrying an SPT::*Ac* construct are white-green variegated (Jones et al., 1989). We can enrich for *Ac* excision from SPT::*Ac* mutable loci by selecting green seedlings from among the predominantly variegated progeny of SPT::*Ac* plants. Most of these green seedlings carry germinal excision alleles (SPT′) and between 50% to 70% of them carry a *trAc*(Jones et al., 1990a, 1991).

The location of the *trAc* relative to the SPT donor locus can be mapped using the scheme shown in Figure 1. In this scheme, use is made of a tester stock carrying a nonautonomous defective element in the SPT gene, which we have termed *Ds* by analogy with maize (Jones et al., 1990a). The SPT::*Ds* allele serves as a tester for the presence of *Ac* in the genome: when germinated in streptomycin, seedlings without *Ac* are white, whereas those with *Ac* are white-green variegated because *Ac* can *trans*-activate excision of *Ds* from the reporter gene.

To detect the presence of a *trAc* and to measure its transposition distance, i.e., its frequency of recombination with the SPT′ empty site, green germinal revertants (SPT′/−; *trAc*?/−) are crossed to the *Ds* reporter stock (SPT::*Ds*). Half of the progeny from this cross will be green (SPT+) on streptomycin. The remaining half will be either all white if *Ac* is absent or a mixture of white and variegated if *Ac* is present, the fraction of variegated seedlings being directly proportional to the distance between SPT and the *trAc*. Thus, if the *trAc* is unlinked to SPT, 50% of the nongreen seedlings will be variegated. Conversely, if the *trAc* is recombinationally inseparable from SPT, as it would be if *Ac* had transposed within the T-DNA, no crossover variegated seedlings will be recovered.

Several features of this scheme need to be considered at the outset.

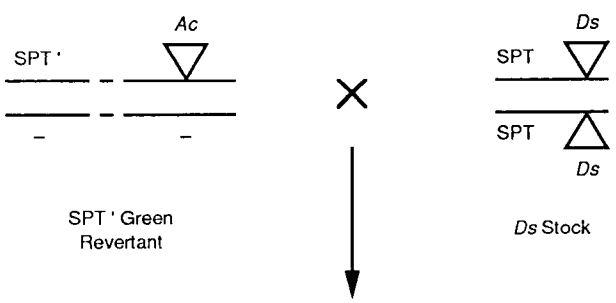
(1) The use of markers flanking the *Ac* mutable allele in maize has allowed an assessment of distance and direction of transposition (Greenblatt, 1984; Dooner and Belachew, 1989). Unlike the two-tailed mapping of *trAc*s in maize, the simple scheme of Figure 1 is one-tailed and allows an estimate of transposition distance, but not of direction.

(2) Similarly, because of the absence of markers flanking the SPT::*Ac* locus, it is not possible to distinguish crossovers from secondary transpositions to unlinked sites, so that only *maximum* genetic distances can be estimated by this scheme. In the one prior case where *Ac* transposition distances in a heterologous host were systematically examined, secondary transposition was found to account for a significant fraction of the variegated progeny only when the apparent recombination frequency was very low (<1%) (Jones et al., 1990a).

(3) The presence of a *trAc* can only be detected genetically by the occurrence of variegated seedlings in the testcross progeny. If the *trAc* still resides within the borders of the T-DNA, it will not be separable from the SPT donor locus by crossing over because the homolog lacks a corresponding region. Therefore, the scheme relies on secondary transposition to detect very closely linked *trAc*s. This shortcoming becomes a problem only when the frequency of secondary transposition is low. Alternatively, *trAc*s can be detected as new *Ac*-hybridizing bands in a DNA gel blot. As will be discussed below, we used a combination of both genetic and DNA gel blot data to determine whether *Ac* was present or absent in several of the green germinal derivatives that failed to produce variegated testcross progeny.

Genetic Analysis

We characterized green SPT′ revertants from four slightly different SPT::*Ac* alleles occupying six different locations in the tobacco genome. The T-DNA region of the four



	Unlinked	Linked
Green	50%	50%
Variegated	25%	<25%
White	25%	>25%

Figure 1. Genetic Scheme Used To Map the Position of the *trAc*s Relative to the SPT Donor Locus.

SPT′ germinal revertants that arose from excision of *Ac* from SPT were crossed to an SPT::*Ds* tester stock. Seed from the cross were germinated on a medium containing streptomycin and the number of green (G), variegated (V), and white (W) seedlings was determined. The maximum distance between SPT and the *trAc* is given by the formula (V/[V + W]) × 100. Secondary transpositions of *Ac* cause an overestimate of genetic distance.

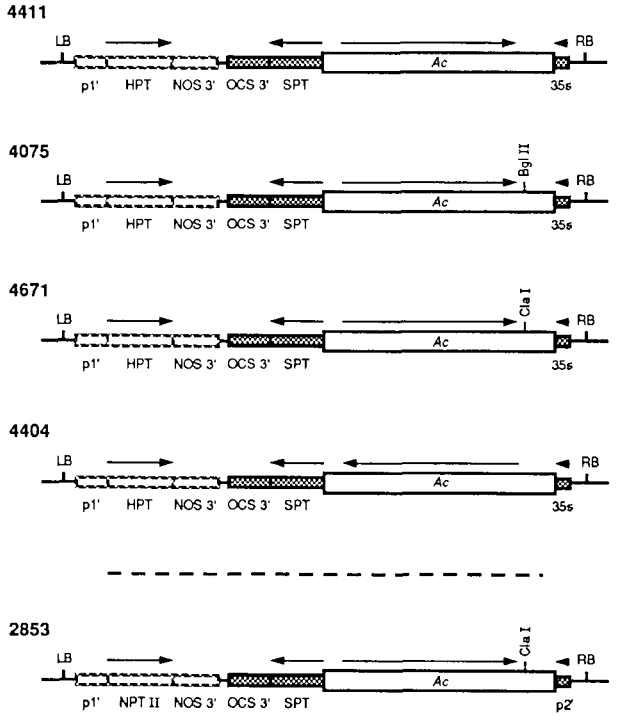


Figure 2. T-DNA Regions of the Four Binary Vector Plasmids Used To Monitor *Ac* Excision from an SPT Gene.

The selectable marker in every case is hygromycin resistance. Plasmids 4411, 4075, and 4671 differ only in the nature of the *Ac* element. *Ac4411* is wild type, *Ac4075* has an introduced BglII site 82 bp 3′ to the mapped polyadenylation site, and *Ac4671* has a ClaI site at that same position. Plasmid 4404 differs from 4411 in the transcriptional orientation of *Ac* relative to the SPT gene. Arrows indicate direction of transcription. The T-DNA region of plasmid 2853, used in our earlier work (Jones et al., 1989, 1990a) is shown for comparison. LB and RB, left and right borders of the T-DNA; p1′ and p2′, promoters from the genes encoding the 1′ and 2′ transcripts of *Agrobacterium*; HPT, hygromycin phosphotransferase; NOS 3′ and OCS 3′, 3′ ends of the nopaline synthase and octopine synthase genes of *Agrobacterium*, respectively; 35S, promoter from cauliflower mosaic virus directing the transcription of the 35S transcript; NPT, neomycin phosphotransferase.

binary vector plasmids harboring the different SPT::*Ac* alleles (4411, 4404, 4075, and 4671) is diagrammed in Figure 2. In all four constructs, an *Ac* element is inserted in the 5′ untranslated leader of a 35S/SPT chimeric gene. The constructs differ in the nature of the *Ac* element and in the transcriptional orientation of *Ac* relative to the SPT gene.

The SPT::*Ac* allele in construct 4411 carries a native *Ac* element in opposite transcriptional orientation to SPT. Construct 4404 differs from 4411 only in the orientation of *Ac* relative to SPT. Construct 4075 differs from 4411 in that the *Ac* element has been modified by the introduction of a BglII cloning site (Jones et al., 1990b) at a position about 82 bp 3′ to the polyadenylation site of the *Ac* transcript (Kunze et al., 1987; Finnegan et al., 1988). Construct 4671 differs from 4075 in that 4 bp have been added at the location of the introduced BglII site to create a ClaI cloning site. This construct is similar to 2853, which we used in earlier work (Jones et al., 1989, 1990a), except that the expression of the SPT gene in 2853 is driven by the 2′ promoter of *Agrobacterium*. The relative frequencies of *Ac* transposition in these constructs are as follows: Ac(+) > Ac-BglII > Ac-ClaI (Jones et al., 1990b; J. Keller, E. Harper, E. Lim, E. Ralston, J. Jones, and H. Dooner, manuscript in preparation).

In this study, we examined the pattern of *Ac* transposition from SPT::*Ac4411* alleles at three different locations and from SPT::*Ac4404*, SPT::*Ac4075*, and SPT::*Ac4671* alleles at one location each. To isolate SPT′ revertants, transgenic tobacco plants homozygous for single SPT::*Ac* inserts were crossed with pollen from untransformed plants. We have accumulated extensive evidence based on DNA gel blots that green SPT′ selections from different capsules of the same plant arise from independent transposition events (J. Keller, unpublished observations). To ensure that each SPT′ selection represented an independent event, we generally analyzed only one green revertant per capsule. When more than one green seedling from the same capsule was selected for analysis, we kept track of each separately, and if the genetic data suggested that they could have arisen from a common premeiotic event (i.e., the SPT-*trAc* estimated distances did not differ significantly from each other), we determined whether they had the same *Ac*-hybridizing band by DNA gel blot analysis.

The overall results of the genetic analysis of 185 SPT′ germinal revertants from six different loci are summarized in Table 1. The SPT′ revertants were crossed to an SPT::*Ds* reporter stock and the progeny seedlings were scored in the presence of streptomycin as being either green, variegated, or white. Although in most families

Table 1. Summary of the Genetic Analysis of SPT′ Revertants from SPT::*Ac* Alleles at Six Different Loci

	trAc Present			
Locus	Linked to SPT	Unlinked to SPT	*Ac* not Detected	Total
4411-A	14	7	15	36
4411-B	18	6	17	41
4411-C	7	6	8	21
4404-A	8	5	8	21
4075-A	12	1	13	26
4671-A	18	5	17	40
Total	77	30	78	185

Table 2. Genetic Analysis of SPT′ Revertants That Have a Linked *trAc*[a]

Locus	SPT′ Revertant	Progeny Segregation[b] G	V	W	V[c] V+W	Locus	SPT′ Revertant	Progeny Segregation[b] G	V	W	V[c] V+W
SPT::*Ac4411-A*	8258.2	151	56	82	0.41	SPT::*Ac4404-A*	8270.3	153	22	107	0.17
	8258.5	155	54	118	0.31		8270.6	127	46	79	0.37
	8259.8	249	31	235	0.12		8271.1	159	1	145	0.007
	8259.9	149	36	133	0.21		8271.1	215	4	197	0.02
	8262.3	78	13	53	0.20		8271.3	193	1	213	0.005
	8262.7	76	1	88	0.01		8271.8	195	2	209	0.01
	8262.9	67	10	48	0.17		8272.7	121	27	88	0.23
	8262.10	102	28	89	0.24		8272.8	248	1	219	0.005
	8263.2	56	16	50	0.24						
	8263.3	431	2	476	0.004	SPT::*Ac4075-A*	8322.1	90	14	68	0.17
	8263.5	116	19	87	0.18		8322.5	264	1	249	0.004
	8263.7	376	2	394	0.005		8322.7	201	17	174	0.088
	8263.9	113	20	88	0.19		8320.7-f1[d]	190	1	160	0.006
	8263.10	150	43	92	0.32		8320.8-f1[d]	130	17	99	0.15
							8320.11	162	60	99	0.38
SPT::*Ac4411-B*	8260.1	168	1	148	0.007		8323.4	235	2	197	0.01
	8260.2	116	9	98	0.08		8324.1-f2[d]	116	3	106	0.03
	8260.3	101	1	58	0.02		8324.2-f2[d]	146	4	143	0.03
	8260.8	237	8	238	0.03		8324.3	206	3	174	0.02
	8260.11	115	1	113	0.009		8324.5-f3[d]	77	1	78	0.01
	8261.3	74	2	81	0.02		8324.6-f3[d]	96	10	61	0.14
	8261.6	270	6	252	0.02						
	8261.8	339	6	314	0.02	SPT::*Ac4671-A*	8330.1	165	10	146	0.064
	8264.3	382	21	388	0.05		8330.3	168	15	145	0.094
	8264.4	528	6	538	0.01		8330.7	117	30	68	0.31
	8264.5	123	7	100	0.07		8330.9	144	28	119	0.19
	8264.6	103	3	134	0.02		8330.11	102	6	110	0.05
	8264.7	549	6	514	0.01		8331.1	267	2	265	0.007
	8265.3	74	4	68	0.06		8331.3	119	6	85	0.07
	8265.8	477	6	479	0.01		8331.5	102	15	79	0.16
	8265.10	165	53	106	0.33		8331.6	88	1	80	0.01
	8266.2	466	98	384	0.20		8331.9	91	3	83	0.03
	8266.9	548	8	560	0.01		8332.1	108	1	91	0.01
							8332.3	96	8	108	0.07
SPT::*Ac4411-C*	8267.1	199	53	107	0.33		8332.4	193	1	198	0.005
	8267.9	212	80	140	0.36		8333.8	90	5	84	0.06
	8268.4	233	4	207	0.02		8333.9	210	2	214	0.009
	8262.6	321	1	352	0.003		8334.1	97	13	73	0.15
	8269.6	225	43	223	0.16		8334.6	115	5	93	0.05
	8269.8	142	45	90	0.33		8334.8	105	5	90	0.05
	8269.9	82	43	75	0.36						

[a] From crosses of the type SPT′/−; *trAc*/− × SPT::*Ds*.

[b] G, green; V, variegated; W, white.

[c] ×100, the maximum SPT-*trAc* genetic distance (in cM).

[d] Pairs of SPT′ derivatives from the same fruit capsule (−f#), determined to have arisen from independent *Ac* transposition events on the basis of either genetic evidence (−f1 and −f3) or DNA gel blot evidence (−f2).

between 200 and 300 individuals were classified, in one family as few as 68, and in others as many as 600 seedlings were examined. Green revertants that produced no variegated progeny were classified as having no genetically detectable *Ac*. Many of these were also characterized molecularly for the presence of a new *Ac*-hybridizing band. Green revertants that produced variegated progeny were classified as having a *trAc*. The ratio of variegated and white seedlings in their progeny was compared by a χ^2 test against the 1:1 ratio expected if the *trAc* was not linked to the SPT′ locus. In families where the observed ratio differed significantly from the expected ratio, the SPT-*trAc* map distances were calculated from the formula $(V/V + W) \times 100$, where V and W represent, respectively, the number of variegated and white seedlings in a family. As discussed earlier, this computation yields an overestimate of the true map distance because *Ac* secondary transpositions and crossovers are grouped together.

Of the 185 SPT′ revertants examined, *Ac* was present in 107 (58%) and not detectable genetically in 78 (42%). Among those SPT′ derivatives in which a *trAc* was present, the *trAc* element was linked to the SPT donor locus in 77 (72%) and unlinked in 30 (28%). The distribution of SPT′ revertants with linked, unlinked, and undetectable *trAc*s was remarkably homogeneous for all six loci ($\chi^2 = 7.98$; 10 *df*; $0.75 > P > 0.50$).

The segregation data for the 77 SPT′ revertants having a *trAc* linked to the donor SPT locus are presented in Table 2. The last column in the table gives the proportion of variegated progeny among the nongreen seedlings in each family. These proportions have been converted into SPT-*trAc* map distances and pooled into classes defined by intervals of 2 map units to derive the distributions of linked *trAc*s for each locus that are shown graphically in Figure 3.

It is readily apparent that the distributions differ from each other. In particular, the distributions of *trAc*s from 4411-A and 4411-B, two loci with sample sizes of 14 and 18 *trAc*s, respectively, appear very different. We used an analysis of variance procedure to compare the distributions statistically. Mean transposition distances were calculated for each locus and compared with each other using the test criterion of least significant differences. The results of this analysis are given in Table 3. The test distinguishes two largely nonoverlapping groups, one consisting of loci 4411-B, 4671-A, 4075-A, and 4404-A, and the other one of loci 4411-A and 4411-C. The means of the loci in one group are significantly different from the means of the loci in the other group, the only exception being 4411-A and 4404-A, which are not found to differ by the test criterion, probably because of the latter's small sample size.

The results of the genetic analysis were somewhat unexpected. The most common transposition pattern of *Ac* in tobacco, exemplified by locus 4411-B, is similar to the pattern of *Ac* transposition seen in the *P* and *bz* loci in maize. There is a pronounced clustering of *trAc*s within 5

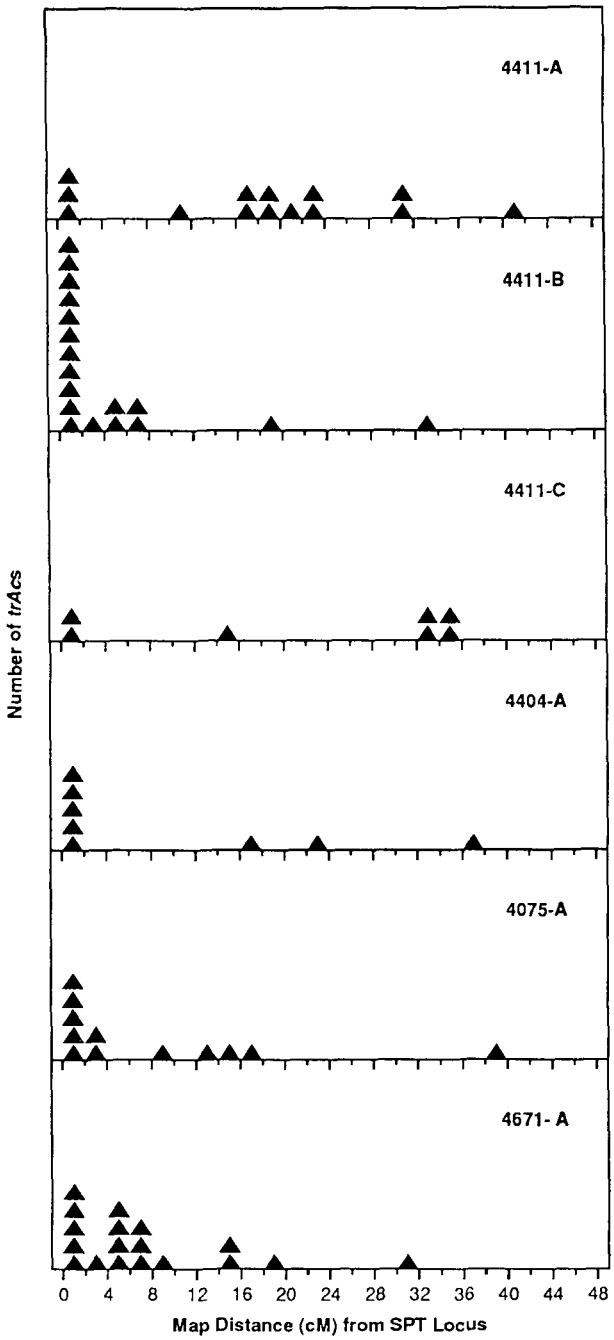


Figure 3. Distribution of *trAc*s among SPT′ Derivatives from Six Different Loci in Tobacco.

Map distances (in centimorgans) between the *trAc* and the donor locus were calculated from the data presented in Table 2.

cM of the donor locus. The transposition pattern of *Ac* exemplified by locus 4411-A, on the other hand, is new. The distribution of *trAc*s about the donor locus is more dispersed and there is a small cluster of *trAc*s about 20 cM away from the donor locus.

One possible explanation for the type of distribution of *trAc*s shown by the 4411-A locus is that many of the intermediate distance values (15 cM to 25 cM) are not real, but reflect, instead, a high frequency of *Ac* secondary transposition. To test whether secondary transpositions contributed heavily to the estimates of SPT-*trAc* genetic distance, we analyzed the DNA of variegated progeny produced by three SPT′ derivatives from 4411-A and one SPT′ derivative from 4411-B whose *trAc*s appeared to map between 20 cM and 24 cM away from the donor locus. Variegated seedlings that arose by crossing over will have an *Ac*-hybridizing band of the same size as the parental SPT′ green selection, whereas those that arose by secondary transposition will have new *Ac*-hybridizing bands. Representative DNA gel blots for two SPT′ derivatives from 4411-A whose *trAc*s map at 24 cM and 21 cM, respectively, are shown in Figure 4. As can be seen from the figure, a majority of the variegated seedlings possess *Ac*-hybridizing bands of the same size as the SPT′ green progenitor, indicating that they arose from crossing over, rather than secondary transposition.

The results of the DNA gel blot analysis are summarized in Table 4. The proportion of variegated seedlings due to crossing over is similar in all four SPT′ derivatives analyzed ($\bar{x} = 0.7$). The estimates of the SPT-*trAc* map distances for these four SPT′ derivatives can be revised on the basis of the DNA gel blot data. The corrected estimates of genetic distance (Table 4, last column) are 18 cM, 16 cM, 13 cM, and 15 cM. We conclude that the intermediate genetic distances for the 4411-A and 4411-B loci are real, although they need to be adjusted down slightly for the

Table 3. Comparison of the Mean Transposition Distances for *trAc*s from the Six Different SPT::*Ac* Loci

Locus	Mean Transposition Distance	N[a]	Statistical Grouping[b]
4411-B	5.4	18	C
4671-A	7.7	18	C
4075-A	8.6	12	C
4404-A	10.2	8	C B
4411-A	18.6	14	B A
4411-C	22.3	7	A

[a] N, number of *trAc*s.
[b] Least significant difference groupings: means with the same letter are not significantly different.

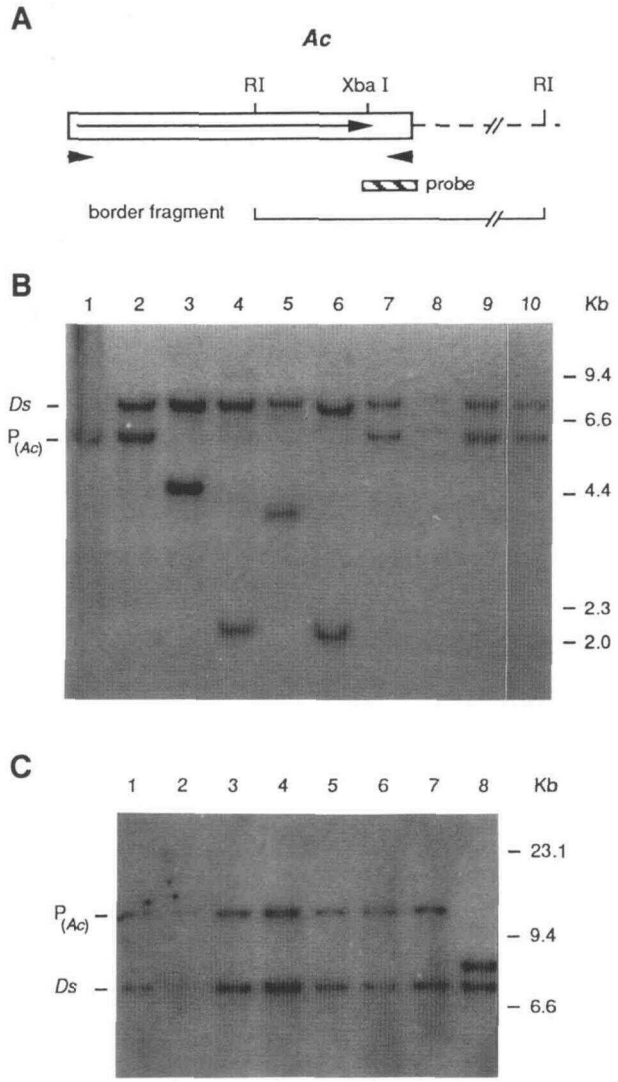


Figure 4. DNA Gel Blot Analysis of Variegated Progeny from Crosses between SPT′ Derivatives and a *Ds* Stock.

EcoRI digests were hybridized with an internal *Ac* probe.
(A) Schematic map of the *Ac* element showing the fixed location of the single EcoRI site in *Ac* and the variable location of the first external EcoRI site in the adjacent tobacco DNA. The *Ac* probe is represented by a hatched box.
(B) Variegated progeny from SPT′ 8262.10 (Table 2). Lane 1, SPT′ 8262.10. Lanes 2 to 10, variegated progeny from 8262.10. The 6.8-kb band is contributed by the SPT::*Ds* allele. Individuals having a parental size band are crossovers (lanes 2, 7 to 10). Individuals with a new size band are interpreted as secondary transpositions.
(C) Lanes 1 to 8, variegated progeny from 8259.9 (Table 2). Individuals 1 to 7 arose by crossing over between the *trAc* (10 kb) and SPT. Individual 8 has a new *Ac* band and probably arose from a secondary transposition event.

Table 4. Summary of the DNA Gel Blot Characterization of Variegated Progeny from the *trAc* Mapping Experiment

Locus	SPT′ Derivative	Crossover Fraction[a]	SPT-*trAc* Distance(cM)	
			Original[b]	Corrected[c]
4411-A	8259.9	7/8	21	18
4411-A	8262.10	14/21	24	16
4411-A	8262.3	7/11	20	13
4411-B	8266.2	3/4	20	15

[a] V seedlings with parental *trAc* band/total number of V seedlings analyzed.
[b] From Table 2.
[c] Recalculated map distance based on DNA gel blot analysis of V progeny ($a \times b$).

contribution of secondary transpositions, and that the transposition patterns of *Ac* from these two loci do, in fact, differ from each other.

Another limitation of the genetic analysis is its potential failure to detect *trAc*s that are very tightly linked to the donor locus and show a low frequency of secondary transposition from their new site. Clearly, this limitation can be overcome by increasing the size of the experiment. In our previous study (Jones et al., 1990a), 14 SPT′ derivatives from the 2853.6 locus—which carries the same *Ac* element as 4671-A—were first characterized for the presence of a new *Ac*-hybridizing band and then analyzed genetically. All 14 produced variegated seedlings in the testcross progeny at some frequency, the lowest frequency being 0.2%. Therefore, the limit of resolution of our present experiment is slightly below that of our earlier experiment.

It is conceivable that some of the green SPT′ revertants with no genetically detectable *Ac* could have a very closely linked *trAc*. This is particularly true for the 4671-A locus whose slightly modified *Ac* element has been shown to transpose at a lower frequency than the other *Ac*s used in the present study and would, therefore, give fewer secondary transposition events in the absence of crossover events (Jones et al., 1989, 1990b; H.K. Dooner, J. Keller, E. Harper, and E. Ralston, unpublished results). To establish whether SPT′ derivatives from 4671-A that lacked a genetically detectable *Ac* also lacked an *Ac*-homologous band, most of them (16/17) were analyzed by DNA gel blots. Only four out of 16 had a new *Ac*-homologous band (data not shown), indicating a possible *trAc*. We conclude that, among excision derivatives from a locus carrying a modified *Ac* element that is slightly impaired in transposition, at most 25% of those scored as having no genetically detectable *Ac* had a very closely linked *trAc*.

Because the unmodified *Ac* element in the 4411 construct is transpositionally more active than the modified element in 4671, we expected the genetic analysis to be at least as sensitive in detecting closely linked *trAc*s from 4411 loci as from 4671 loci. We analyzed SPT′ derivatives from the 4411-A locus that had been classified as lacking a genetically detectable *Ac* (Table 1) and confirmed that, as anticipated, most of them also lacked a new *Ac*-homologous band (data not shown).

In summary, we have exploited the ease and convenience of the genetic test shown in Figure 1 to establish *Ac* transposition patterns for several loci in tobacco and have substantiated the main conclusions drawn from the genetic data through a molecular analysis of several transposition derivatives.

DISCUSSION

We have examined the pattern of transposition of the maize element *Ac* from six different loci in the tobacco genome. In a previous study (Jones et al., 1990a), we found that 71% of all *trAc*s mapped within 1 cM of another donor locus in tobacco, the 2853.6 locus. Although the clustering of *trAc*s about the 2853.6 locus was in general agreement with earlier work in maize, the tightness of the linkage between the donor locus and the great majority of *trAc* receptor sites created uncertainty about the potential usefulness of *Ac* as a transposon tag in heterologous systems (Wessler, 1990).

The results of the current study dispel those doubts. None of the six loci examined showed as tight a clustering of *trAc* receptor sites around the donor locus as 2853.6. One possible explanation for our earlier result was that the *Ac* element in the 2853 construct had been modified by the introduction of a ClaI site near the 3′ end of *Ac*. However, one of the loci analyzed here, 4671-A, carried the same *Ac*-ClaI element as 2853.6 but did not show the same exaggerated clustering of *trAc* receptor sites. Possibly, our earlier observations were due to the inverted repeat of the T-DNA insert in the 2853.6 locus (Jones et al., 1989, 1990a), which contrasts with the single T-DNA in the six loci studied here. If the results we report here for tobacco hold true for other dicot species, such as *Arabidopsis*, *Petunia*, and tomato, *Ac* should be a useful transposon for tagging genes in heterologous systems.

An interesting finding from the present study is that the pattern of *Ac* transposition to linked sites in tobacco may vary from locus to locus. Four of the six loci analyzed give patterns that are generally similar to the pattern reported previously in maize for the *P* and *bz* loci (Greenblatt, 1984; Dooner and Belachew, 1989):a majority of the linked *trAc*s are clustered within 5 cM of the donor locus. However, the other two loci give a different pattern: instead of being clustered around the donor locus, the linked *trAc*s are more dispersed. In one of these loci, 4411-A, there is a small cluster of *trAc*s 15 cM to 20 cM away from the donor locus. Although the four constructs used in the present

investigation differed in the nature of the *Ac* element used and/or in the transcriptional orientation of *Ac* relative to the SPT gene (Figure 2), the differences in transposition patterns cannot be attributed to either of these factors. For example, loci 4411-B and 4411-A, which carry the same T-DNA construct, illustrate the clustered and dispersed patterns, respectively. On the other hand, loci 4411-B, 4671-A, and 4075-A, which carry slightly different *Ac* elements, give similar transposition patterns.

In all likelihood, the pattern of *Ac* transposition from a locus is a property of the locus itself, i.e., a function of the chromatin organization in the vicinity of the locus. In the case of transgenic tobacco, it would be a property of the specific site of integration of the T-DNA in the tobacco genome. It is conceivable that in maize more than one *Ac* transposition pattern also occurs but has not been detected yet because of the small number of loci analyzed to date.

What properties of the chromatin in the vicinity of the donor locus could possibly account for the different distributions of *trAc*s? In an attempt to answer this question, we need to consider what is known about *Ac* transposition.

First, the clustering of *trAc*s about the donor locus represents the most common *Ac* transposition pattern in tobacco and in maize. The simplest interpretation of this pattern is that the *Ac* transposase does not generate a free, extrachromosomal intermediate but instead mediates the formation of transposition complexes between the *Ac* donor site and competent receptor sites that are physically nearby and that, therefore, will tend to be linked in the same chromosome. A physical association between donor and receptor sites has been invoked by Brink and Williams (1973) and Greenblatt (1984) to explain the pattern of *Ac* transposition from the *P* locus in maize and by Robbins et al. (1989) to explain an unusual chromosome rearrangement involving *Tam3* in *Antirrhinum*. Second, the analysis of pericarp twin sectors in maize has led to the model that *Ac* transposes during chromosome replication from a replicated donor site to a receptor site that may be either unreplicated or replicated (Greenblatt and Brink, 1962). Transposition of *Ac* to an unreplicated site will result in an over-replication of *Ac* in that particular cell division. A molecular examination of the *trAc*s in maize pericarp twin sectors has provided confirmatory evidence that *Ac* can transpose to unreplicated sites (Chen et al., 1987).

We propose that competent receptor sites must reside in replicons that undergo replication at the same time in S phase as the donor site. Greenblatt (1984) has also suggested that the replication state of a chromosomal segment may affect its ability to serve as a *trAc* receptor site. The existence of replicon families that replicate DNA at a given time during S phase has been clearly established in higher plants (Van't Hof, 1988). Within each replicon family there are clusters or groups of replicons arranged end-to-end along the DNA duplex that replicate DNA nearly simultaneously. The clustering of *trAc*s about donor loci commonly observed in maize and tobacco may be a reflection of this level of replicon organization.

The rarer, more dispersed *Ac* transposition pattern would be produced by *Ac* donor sites that replicate their DNA at a different time from most of the neighboring DNA. Such would be the situation of replicons that are members of small clusters or are not organized into clusters and that replicate their DNA at a different time in the S phase from neighboring clusters.

An alternative explanation for the contrasting patterns of *Ac* reinsertion observed in this study is that they are due to dramatic local differences in the relationship between physical and genetic distance from one region of the genome to another. We do not favor this model because it fails to take into consideration what is known about the distribution of recombination events in the genome and the nature of the DNA into which *Ac* transposes.

It has been proposed that recombination in eukaryotic genomes may be confined to genes (Thurieaux, 1977) and data from maize support this concept. When the genetic and molecular maps of several genes are compared, it is clear that the frequency of recombination per kilobase within genes is as much as 100 times higher than the average for the entire genome: *bz* (Dooner et al., 1985; Dooner, 1986); *wx* (Nelson, 1968; Wessler and Varagona, 1985); *Adh* (Freeling, 1977; Sachs et al., 1986) and *R* (J.L. Kermicle, personal communication). The concept that most, if not all, eukaryotic recombination is intragenic implies that the genetic distance between two sites is proportional to the number of genes in the corresponding interval and that the differences in the relationship between physical and genetic lengths throughout the genome represent differences in gene density among different regions.

There is evidence that *Ac* transposes preferentially to genes and, thus, into regions of the genome contributing to genetic length. This evidence derives from the findings that *Ac* tends to transpose into hypomethylated DNA (Chen et al., 1988) and that it is this rare fraction of total DNA that is associated with genes in higher plants (Antequera and Bird, 1988).

To account for the dispersed distribution of *trAc*s, one has to explain why regions of DNA adjacent to the T-DNA that are recombinationally active and, hence, contain genes, do not always serve as receptor sites for *Ac*. We believe that the model based on timing of replication of the adjacent DNA accomplishes this better than a model based purely on random fluctuations of the ratio between genetic and physical distances across the genome.

The practical implications of our findings for *Ac* tagging experiments in heterologous species are that systems based on *Ac* are likely to succeed if *Ac* behaves in those species as it does in tobacco and in maize and that the probability of success in directed tagging experiments, i.e., those that aim to tag a *specific* gene rather than any interesting gene in the genome, will be enhanced largely if *Ac* is linked in *cis* to the desired target locus.

METHODS

Constructs

The binary vector plasmids that were used in our experiments were constructed by J. Jones (DNA Plant Technology, Oakland, CA). They are shown diagramatically in Figure 2 and will be described in more detail elsewhere (J. Keller, E. Harper, E. Lim, E. Ralston, J. Jones, and H. Dooner, manuscript in preparation).

Plant and Bacterial Genotypes

All work was carried out with *Nicotiana tabacum* var Petite Havana, which is naturally sensitive to streptomycin. The SPT::*Ac* plants used in our experiments were obtained by transformation with *Agrobacterium tumefaciens* LBA4404 (Hoekema et al., 1983) carrying the various binary vector plasmids. The derivation of the *Ac* tester line carrying a *Ds* element in the SPT gene has been described (Jones et al., 1990a).

Selection of SPT′ Revertants

SPT′ derivatives were isolated as green seedlings in streptomycin-containing plates as described previously (Jones et al., 1989, 1990a).

DNA Extraction and Analysis

Leaves from plants at the six- to- seven leaf stage were ground up with a glass homogenizer in buffer consisting of 0.14 M sorbitol, 0.22 M Tris, pH 8, 0.022 M EDTA, 0.8 M NaCl, 0.8% cetyl trimethylammonium bromide, and 1% lauryl sarcosine. The leaf homogenates were heated to 65°C for 20 min and extracted with chloroform. The nucleic acids in the aqueous phase were precipitated with isopropyl alcohol. Approximately 5 μg of each DNA sample were digested with EcoRI, separated through 0.9% agarose, and blotted to Duralon-UV (Stratagene). The blots were probed with a 1-kb *Ac* riboprobe, corresponding to an Xba-ClaI fragment from the 5′ end of *Ac* in the plasmid pJAC3 (generously provided by John Yoder, University of California, Davis, and Peter Starlinger, University of Cologne, Germany). The location of the probe within the transposon is shown in Figure 4.

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