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Preferential transposition of *Ac* to linked sites in Arabidopsis

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Abstract. We have investigated the pattern of transposition of an intact, 4.6-kb *Ac* element in *Arabidopsis thaliana*. Because the *trans*-acting transposition function (transposase) of *Ac* is not fully penetrant in Arabidopsis, it is not possible to use it as a diagnostic feature to score *Ac* genetically, as has been done in maize and tobacco. Instead, the presence or absence of a transposed *Ac* (*trAc*) was monitored by Southern blots. Germinal transpositions from the marker SPT::*Ac* were selected using a streptomycin germination assay and scored for the presence of a *trAc*. Segregation of the *trAc* element and the SPT donor locus was scored in the F_2 progeny of the germinal revertants, and the recombination fraction between the *trAc* element and SPT was estimated by the method of maximum likelihood. We have found that, as in maize and tobacco, receptor sites for *trAc*s in Arabidopsis tend to be linked to the *Ac* donor locus.

Key words: Transposable elements – *Activator* – Maize – Arabidopsis – *Mapping*

Introduction

Knowledge of the transposition pattern of a transposable element in a given species is valuable in designing gene tagging experiments. The maize element *Activator* (*Ac*) transposes to closely linked sites in its natural host (Greenblatt 1984; Dooner and Belachew 1989), so the probability of tagging a particular gene with *Ac* increases if the target gene and the *Ac* element are linked. Dellaporta et al. (1988) exploited this feature of *Ac* transposition to tag, and eventually isolate, the *R* gene in the short arm of chromosome 10 with an *Ac* element that was linked to *R*.

Ac has now been shown to transpose in a number of heterologous plant species (for a review, see Haring et al. 1991). In tobacco, the receptor sites for transposed *Ac* (*trAc*) elements tend to be linked to the donor locus, as in maize, although the actual distribution of linked receptor sites seems to be locus specific (Jones et al. 1990; Dooner et al. 1991). In tomato, the receptor sites are distributed in small clusters that may or may not be linked to the donor locus (Osborne et al. 1991; Belzile and Yoder 1992). Secondary transpositions, which appear to be common in tomato because of *Ac*'s high transposition activity, probably contribute to this pattern.

Arabidopsis thaliana has become an attractive model system for the genetic analysis of plant processes because of its diploidy, small genome size and rapid life cycle (Meyerowitz 1989). Following the demonstration that *Ac* transposes in Arabidopsis (van Sluys et al. 1987), several laboratories have worked on developing *Ac*-based transposon tagging systems for gene isolation in that species (Schmidt and Willmitzer 1989; Dean et al. 1992; Greveldding et al. 1992; Keller et al. 1992; Swinburne et al. 1992). Since the efficiency of an experiment designed to tag a particular gene with *Ac* will be greatly affected by the pattern of transposition of *Ac*, we decided to examine the distribution of *trAc* receptor sites in Arabidopsis. However, because of the low somatic transposition activity of *Ac* in Arabidopsis (Dean et al. 1992; Keller et al. 1992), phenotype is not a good indicator of *Ac* genotype. This makes it necessary to adopt a different strategy for mapping *trAc*s from the one used in maize and tobacco, which is based on *Ac*'s ability to *trans*-activate the excision of a transposition-defective (*Ds*) element from a reporter gene. We have used Southern blot analysis to

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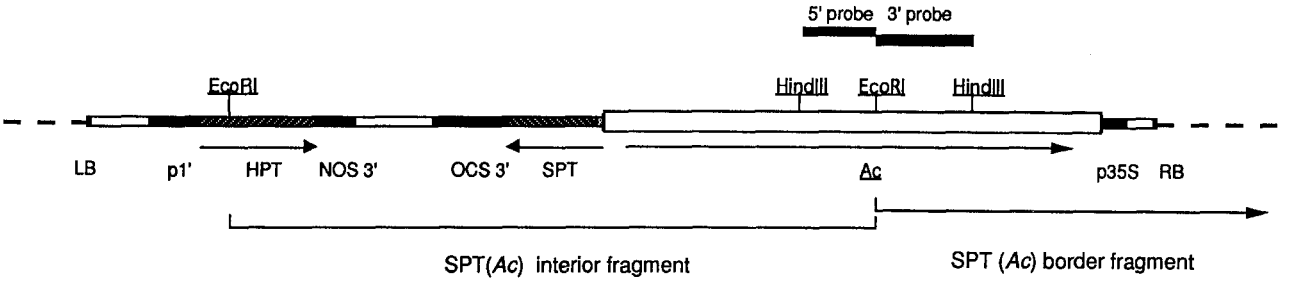


Fig. 1. Map of the T-DNA region containing the SPT::*Ac* gene. A schematic diagram of the T-DNA in transformants C201 and C231 is shown, along with the position of the 5′ and 3′ *Ac*-specific probes used in the analysis. Flanking plant chromosomal DNA is indicated by the *dashed lines*. The *Ac* element in transformants B222 and B236 is in the opposite orientation to that shown. The SPT::*Ac* internal *Eco*RI fragment is 5.9 kb for C201 and C231 (shown *below* the map) and 5.6 kb for B222 and B236. The SPT::*Ac* border fragment varies with the transformant and extends to the first *Eco*RI site in the flanking plant DNA. *p1′* the T-DNA 1′ promoter; *HPT* hygromycin phosphotransferase; *SPT* streptomycin phosphotransferase; *NOS3′* and *OCS3′* nopaline and octopine synthase polyadenylation sequences, respectively; *p35S* the CaMV 35S promoter; *LB* and *RB* left and right borders of the T-DNA, respectively

score for the presence or absence of *trAc* elements among segregating F_2 progenies from individuals that had undergone transposition and have mapped the location of the *trAc* relative to its donor locus by the method of maximum likelihood. We conclude from this study that *Ac* tends to transpose to sites linked to the donor locus in Arabidopsis, as it does in maize and tobacco.

Materials and methods

Constructions

The binary vectors carrying the excision marker SPT::*Ac* have been described elsewhere (Keller et al. 1993). The 35S/*Ac* element used to *trans*-activate *Ac* in transformant B236 contains an *Ac* coding region that has the N-terminal 102 amino acids removed. It was designed to test whether the observation in petunia (Houba-Herin et al. 1990) that a similarly truncated transposase gene was more active than the full length gene held for Arabidopsis. The 35S/*Ac* element was constructed by changing nucleotides 1398 and 1399 preceding the tenth ATG in the *Ac* element from ATATGG to CCATGG to create a *Nco*I site. This was accomplished by polymerase chain reaction (PCR) amplification of the sequences from the tenth ATG to the *Eco*RI site. This fragment was cloned into pCAB22L-CH (Harpster et al. 1989), and the remaining 2 kb of the *Ac* element was cloned into the construct as an *Eco*RI fragment from pJJ4361 (Keller et al. 1993). The 35S/*Ac* gene was then retrieved from the vector as a 4.1-kb *Eco*RI (partial) to *Bgl*II fragment and cloned between the *Bam*HI and *Eco*RI sites of pAGS 502 (Jones et al. 1992). pAGS 502 has a NOS/NPTII gene for selection of kanamycin-resistant plant transformants.

Transformation, excision assay and crosses

The transformation procedure, the streptomycin excision assay and the characterization of *Ac* activity in the Arabidopsis transformants have been described previously (Keller et al. 1992). Green, resistant seedlings were selected in streptomycin among the self-progeny of C201, B222 and C231. Transformant T1403, which contains the 35S/*Ac* element used to *trans*-activate B236, has one T-DNA insert as determined by DNA blot border fragment analysis and segregation on kanamycin. This transformant was crossed to B236, the F_1 was allowed to self-pollinate and the F_2 seed was plated on streptomycin to select green, resistant seedlings.

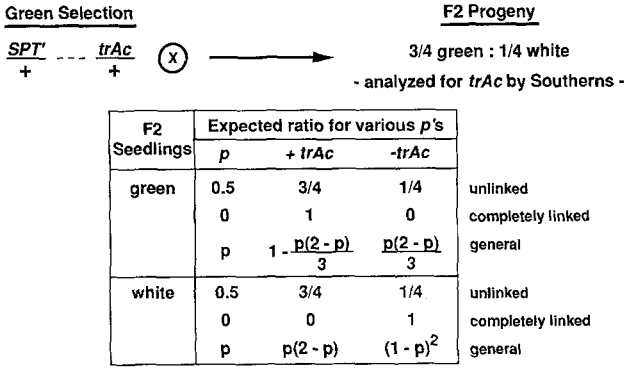
DNA analysis

The procedures for DNA extraction from individual *Arabidopsis* plants and for Southern blot analysis are given in Keller et al. (1992a).

Results and discussion

The streptomycin assay for *Ac* excision (Jones et al. 1989) has previously been described for use in Arabidopsis (Dean et al. 1992; Keller et al. 1992). For the assay, transformants are generated that have an *Ac* element interrupting a chimeric streptomycin phosphotransferase (SPT) gene engineered for expression in plants. Untransformed seedlings bleach when germinated in the presence of streptomycin, but some of the seedling progeny of SPT::*Ac* transformants appear green-white variegated as a consequence of somatic excisions of *Ac* during cotyledon development. Fully green streptomycin-resistant seedlings arise from *Ac* excisions in the germ line of the SPT::*Ac* parent. They all carry germinal excision alleles (SPT′), and about half carry *trAc* elements, as determined by DNA blot analysis.

The transformants used in this study, C201, B222, C231 and B236, have been characterized in detail for T-DNA copy number and *Ac* activity (Keller et al. 1992). In C201 and C231, *Ac* is inserted into the SPT gene in the orientation shown in Fig. 1, in B222 and B236, *Ac* is inserted in the opposite orientation. C201 has three SPT::*Ac* genes at one locus and gives many streptomycin-resistant progeny. B222 and C231 each have one *Ac* element that occasionally excises germinally. B236 has one SPT::*Ac* insert that is germinally inactive by itself but can excise if transposase activity is added in trans using a p35S/*Ac* element, i.e., a chimeric gene in which the *Ac*



F2 Seedlings	Expected ratio for various p's			
	p	+ trAc	-trAc	
green	0.5	3/4	1/4	unlinked
	0	1	0	completely linked
	p	$1-\frac{p(2-p)}{3}$	$\frac{p(2-p)}{3}$	general
white	0.5	3/4	1/4	unlinked
	0	0	1	completely linked
	p	p(2 - p)	$(1-p)^2$	general

Fig. 2. Procedure for linkage analysis of *trAcs* in Arabidopsis

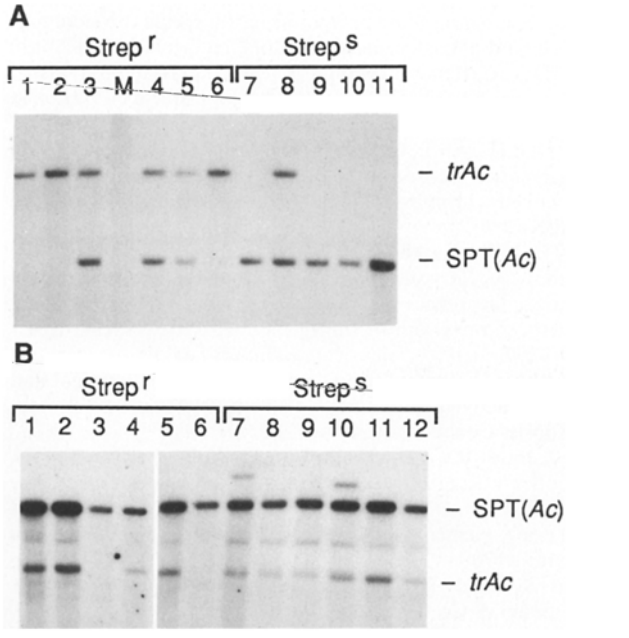


Fig. 3A, B. Southern blot segregation analysis for *trAcs*. Individual green, (streptomycin resistant) and white (streptomycin sensitive) F_2 progeny from green selections carrying a *trAc* element in the genome were analyzed. *Eco*RI-digested DNA was blotted and hybridized to the *Ac* probes shown in Fig. 1. **A** Analysis of *trAc9*, a linked element. *Lanes 1–6* DNA from resistant progeny; *lanes 7–11* DNA from sensitive progeny, *M* marker lane. The band corresponding to the *trAc* element is present in all of the resistant progeny and in one of five sensitive progeny. **B** Analysis of *trAc3*, an unlinked element. *Lanes 1–6* DNA from resistant progeny, *lanes 7–12* DNA from sensitive progeny. The *trAc* band is present in four of six resistant and six of six sensitive progeny

transposase is expressed behind a cauliflower mosaic virus 35S promoter (see Material and methods).

The *trAcs* were mapped relative to their donor SPT′ sites by testing for cosegregation of the streptomycin-resistant trait and the *trAc* in the progeny of the green selections. The analysis was carried out by first allowing the green selections to self-pollinate and then germinating the resulting F_2 seeds in the presence of streptomycin. Resistant and sensitive F_2 progeny were selected, grown to the bolting stage and genotyped by DNA blot analysis. Leaf DNA from each F_2 plant was digested with *Eco*RI, which cuts once within *Ac*, blotted and probed with the 5′ and/or 3′ end *Ac* probes shown in Fig. 1. The size of the *Eco*RI fragment(s) detected in this way depends on the DNA flanking the *Ac* element and differs for the SPT::*Ac* allele and the various *trAcs*. Bands not corresponding to the *trAc* element being mapped, which could have arisen from secondary transpositions, were rarely seen in the segregating F_2 progenies. Therefore, secondary transpositions of *Ac* do not complicate the mapping.

Table 1. Mapping of *trAcs* by Southern blot analysis

trAc	SPT::*Ac* locus	Fraction of progeny with *trAc*		χ^2 analysis	p^a
		Green	White		
trAc1	C201	12/17	10/10	Unlinked	(0.5)
trAc3	C201	17/20	6/6	Unlinked	(0.5)
trAc4	C201	14/18	4/11	Linked	0.25
trAc5	C201	20/20	1/6	Linked	0.04
trAc8	B222	10/12	6/9	Unlinked	(0.5)
trAc9	C231	9/9	2/9	Linked	0.09
trAc10	B236	4/6	0/7	Linked	0.10
trAc11	B236	6/6	0/9	Linked	0

[a] p = recombination fraction; $p \times 100$ = map distance between SPT′ and *trAc* in cM

The linkage analysis is outlined in Fig. 2. The expected outcome for a *trAc* which is inseparable from the SPT′ donor site (i.e., p, the recombination fraction, equals 0) is for all of the green, streptomycin resistant progeny, but none of the white, streptomycin sensitive progeny, to carry the *trAc*. An example of the pattern given by a *trAc* element that is closely linked, although separable from the SPT′ site (*trAc9* in Table 1), is shown in Fig. 3A. If the *trAc* and SPT′ are unlinked ($p = 0.5$), then 75% of both streptomycin-resistant and sensitive progeny should carry the *trAc* band. An example of the unlinked pattern (*trAc3* in Table 1) is shown in Fig. 3B. Presence or absence of linkage was determined by a χ^2 test (1 *df*) that compared the joint segregation of the *trAc* and the donor locus against the expectation of independence. Estimates of p, the recombination fraction between the *trAc* element and the SPT′ donor locus, were obtained by the method of maximum likelihood (Mather 1951) using the SAS (1985) statistical package.

The results of the mapping analysis are given in Table 1. DNA from 191 F_2 progeny from eight different green selections carrying a *trAc* was analyzed by Southern blots. The tedious nature of the assay precludes the generation of more extensive data for the transposition of

unmodified *Ac* elements. Of the eight *trAc*s tested for linkage to the SPT′ donor site, five were linked and three were unlinked to the donor locus. This ratio of linked to unlinked *trAc*s (0.62) is not different from that observed in maize and tobacco. Among the five linked elements, the distance between the SPT donor locus and the *trAc* element ranged from 0 to 25 cM, but four of the five *trAc*s were within 10 cM from SPT. We conclude from these limited data that *Ac* also tends to transpose to sites linked to the donor locus in Arabidopsis, as it does in maize and tobacco. A less laborious approach to mapping the location of transposed elements would involve the development of marked *Ds* type elements (Bancroft et al. 1992, Grevelding et al. 1992) to facilitate the scoring of plants carrying the transposed element.

Our results validate the directed tagging approach, which has been used in maize (Dellaporta et al. 1988), for tagging genes with *Ac* in Arabidopsis. In order to tag a specific gene it is desirable to initiate the tagging experiment with an *Ac* element linked to the locus of choice. Though setting up the stocks may be laborious, in the long run a collection of Arabidopsis stocks having *Ac* at mapped locations in the genome should prove of great utility in gene isolation experiments.

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