The Plant Cell, Vol. 10, 427–434, March 1998 © 1998 American Society of Plant Physiologists

The Transposition Frequency of *Tag1* Elements Is Increased in Transgenic Arabidopsis Lines

Anuj M. Bhatt,[a,1] Clare Lister,[a] Nigel Crawford,[b] and Caroline Dean[a,2]

[a] Department of Molecular Genetics, John Innes Centre, Colney Lane, Norwich NR4 7UH, United Kingdom
[b] Department of Biology, University of California, San Diego, La Jolla, California 92093

***Tag1* was identified as a highly active endogenous transposable element in transgenic *Arabidopsis thaliana* Landsberg *erecta* plants carrying the maize transposable element *Activator* (*Ac*). Here, we describe experiments designed to determine the basis for the high activity of *Tag1*. The frequency of transposition of *Tag1* elements was compared in lines containing or lacking *Ac* transposase to assess the effect of *Ac* transposase on *Tag1* activity. Three populations of nontransgenic plants, including nontransformed regenerants, were also analyzed. The high level of activity of *Tag1* did not correlate with the presence or absence of *Ac* transposase but was significantly higher in transgenic lines. This result was maintained through at least six generations after transformation. These data suggest that *Tag1* transposition is stimulated by processes that occur during the Agrobacterium transformation and that thereafter remain active. Two *Tag1* elements are tightly linked in the Landsberg *erecta* genome and map to the lower arm of chromosome 1. *Tag1* elements were found in only a few *A. thaliana* ecotypes but were present in four other *Arabidopsis* species.**

INTRODUCTION

A number of mobile elements have been identified in *Arabidopsis thaliana.* Two of these, members of the *Ta* family (Voytas and Ausubel, 1988; Voytas et al., 1990; Konieczny et al., 1991) and *Athila* (Pelissier et al., 1995), are retrotransposon elements that use RNA intermediates in the transposition process. Until recently, the only other transposon characterized in *A. thaliana* was the *Tat1* element (Peleman et al., 1991); however, it is still unclear whether *Tat1* is an active element. Lack of a well-characterized transposable element system in *A. thaliana* led a number of groups to introduce heterologous elements into *A. thaliana* to establish a transposon-tagging system (Bancroft et al., 1992; Dean et al., 1992; Swinburne et al., 1992; Sundaresan et al., 1995). During the screening for *Activator* (*Ac*)-induced mutations in a population of Landsberg *erecta* plants, an unstable chlorate-resistant mutation (*chl1-6*) was identified. Gel blot analysis of DNA from *chl1-6* with the previously cloned *CHL1* gene (Tsay et al., 1993a) showed that it carried a 3.3-kb insertion, which is shorter than expected for an *Ac* element. Subsequent analysis identified the insertion as a new transposable element that was termed *Tag1* (for tagging *A. thaliana* genes; Tsay et al., 1993b).

Tag1 had inserted into an intron in the *CHL* gene and generated an 8-bp duplication of the target site. This is a feature shared by *Ac* elements (Fedoroff et al., 1983). The element had 22-bp terminal-inverted repeats that contained A and G residues at positions 2 and 5 in the left repeat and complementary T and C residues in the right repeat. These residues are conserved in the short inverted repeats of the *hAT* superfamily of transposable elements (Warren et al., 1994). Somatic excision of *Tag1* from the 5′ untranslated region of a cauliflower mosaic virus 35S promoter fusion driving β-glucuronidase demonstrated that *Tag1* is an autonomous element in *A. thaliana* and tobacco (Frank et al., 1997). Recently, *Tag1* has been used to tag a gene involved in organ separation in *A. thaliana* (Aida et al., 1997).

Tag1 was very active in the transgenic line in which it was first identified, excising from *CHL1* to produce chlorate-sensitive revertants at a frequency of ∼30% (Tsay et al., 1993b). Unstable mutations have not been commonly observed over many years of genetic analysis in the *A. thaliana* Landsberg *erecta* background; therefore, the high activity of *Tag1* is surprising. We decided to investigate the activity of *Tag1* elements in a large number of independent lines to determine whether *Ac* transposase affects *Tag1* activity and whether *Tag1* elements are equally active in nontransgenic and transgenic lines.

Tag1 is present in two copies in the Landsberg *erecta* genome but is not present in the other commonly used ecotypes, Columbia and Wassilewskija (Ws; Tsay et al., 1993b; Frank et al., 1997). To extend the use of *Tag1* for tagging purposes, we have mapped *Tag1* elements onto the restriction fragment length polymorphism map and analyzed

[1] Current address: Department of Plant Sciences, University of Oxford, South Parks Road, Oxford OX1 3RB, UK.
[2] To whom correspondence should be addressed. E-mail caroline.dean@bbsrc.ac.uk; fax 44-1603-505725.

the distribution of the element in different *A. thaliana* ecotypes and different *Arabidopsis* species.

RESULTS

Comparison of *Tag1* Activity in Transgenic Lines Expressing or Not Expressing *Ac* Transposase

The Landsberg *erecta* transformants used to generate lines to study *Tag1* element activity are summarized in Table 1. Transgenic lines expressing *Ac* transposase were generated from five transformants containing *Ac* elements cloned into the 5′ untranslated region of a streptomycin resistance fusion (*SPT*::*Ac* and *SPT*::*AcΔNaeI* constructs) plus three transformants containing a stabilized *Ac* carrying a tobacco mosaic virus Ω 5′ untranslated leader (*sAcΩ*). *sAcΩ* was made by cloning the Ω sequence into the NaeI site of an *ΔNaeIsAc* element (S. Scofield, unpublished data). The Ω sequence has been shown to improve the translational efficiency of different mRNAs (Gallie et al., 1987). Seventy-eight transformants containing a *Dissociation* (*Ds*) element carrying a hygromycin resistance fusion (*Hm^RDs*) represented transgenic lines not expressing (or never having contained) *Ac* transposase. Transgenic lines containing DNA completely unrelated to *Ac* or *Ds* sequences were represented by 50 transformants containing *FCA* transgenes.

Table 1. Transgenes Contained in the Landsberg *erecta* Transgenic Lines Used in the Analysis of *Tag1* Element Activity

Transgene[a]	Element	Reference
SPT::*Ac*	Wild-type *Ac* in an *SPT* marker[b]	Dean et al. (1992)
SPT::*AcΔNaeI*	NaeI-deleted *Ac* in an *SPT* marker	Lawson et al. (1994)
sAcΩ	*Ac* lacking a 3′ terminus with Ω 5′ UTL[c]	S. Scofield, unpublished data
Hm^RDs	*Ds* carrying *HPT* in an *SPT* marker[d]	Bancroft et al. (1992)
FCA transgenes 35S–transcript β 35S–transcript δ *FCA*–transcript γ *FCA–GUS*	None	Macknight et al. (1997); R. Macknight and C. Dean, unpublished data

[a] All of the T-DNAs contained a T_R 1′ promoter (Velten et al., 1984) and neomycin phosphotransferase II fusion to select for kanamycin-resistant transformants.
[b] *SPT* is a cauliflower mosaic virus 35S promoter–streptomycin phosphotransferase fusion conferring resistance to streptomycin.
[c] UTL, untranslated leader.
[d] *HPT* is a cauliflower mosaic virus 35S promoter–hygromycin phosphotransferase fusion conferring resistance to hygromycin.

Transposition frequency of *Tag1* elements was monitored using DNA gel blot analysis. Progeny of independent transformants carrying the different constructs were assayed at different generations after transformation. DNA was made from individual plants or pooled progeny (at least 10 individuals) and digested with either SspI or HindIII. A germinally inherited transposition event was scored as the presence of a new *Tag1* element elsewhere in the genome (as determined by the presence of differently sized restriction fragments hybridizing with a *Tag1* probe). *Tag1* excisions, that is, loss of *Tag1*-hybridizing fragments, were not scored as transposition events to avoid counting one transposition event twice. However, if excision was not associated with a reinsertion event, then the number of *Tag1* transposition events would have been underestimated.

Table 2 shows a comparison of the frequency of novel, independent *Tag1* insertions in progeny from different transgenic lines. When a *Tag1* transposition event was common to several siblings due to an early transposition event in the parent, this was only counted once. The percentage of plants carrying *SPT*::*Ac*, *SPT*::*AcΔNaeI*, *sAcΩ*, and *Hm^RDs* constructs or *FCA* transgenes that contained a novel *Tag1* insertion varied from 10 to 100%. The *Tag1* transposition frequencies observed in these transgenic populations are in line with the 30% reversion frequency observed for the original chlorate-resistant mutant (Tsay et al., 1993b). There was no obvious relationship between the frequency of *Tag1* transposition and the activity of the different *Ac* derivatives. The four *SPT*::*AcΔNaeI* transformants showed an average germinal *Ac* excision frequency of 2.7% (Lawson et al., 1994), whereas *SPT*::*Ac* transformants showed average frequencies five to 10-fold lower (Dean et al., 1992).

The three transformants carrying an *sAcΩ* construct all showed a novel *Tag1* reinsertion, making them the class of transformants showing highest *Tag1* activity. The frequency of germinal *Ds* transposition when these *sAcΩ* transformants were crossed to a *Ds* tester line was similar to that when the *Ac* transposase was provided by *ΔNaeIsAc* (Bancroft et al., 1992; A. Bhatt and C. Dean, unpublished results) and was in the range of 1 to 6%. T_3 progeny from transformants carrying the *Hm^RDs* elements, which had not been crossed to plants containing an *Ac* transposase source, showed levels of *Tag1* activity similar to lines carrying the autonomous *Ac* elements. Similar levels of activity were also observed in T_3 progeny from lines carrying *FCA* transgenes. Thus, the level of *Tag1* activity is reproducibly high in the range of transgenic populations analyzed, but the high activity is not dependent on the presence of *Ac* transposase or *Ac*/*Ds* sequences.

Tag1 Activity in Nontransgenic Lines

Two hundred and ninety-eight recombinant inbred (RI) lines from a cross between Landsberg *erecta* and Columbia were also analyzed for *Tag1* activity. These lines had not experi-

Table 2. Comparison of the Frequency of Novel, Independent *Tag1* Insertions in Different Plant Populations

Plant Line[a]	Generation Analyzed[b]	No. of Plants	No. of Independent *Tag1* Insertions[c]	Frequency of New *Tag1* Insertions (%)
SPT::*Ac*-A17	T_7	57	10	18
SPT::*AcΔNaeI*-A3	$T_6 + T_7$	5	3	60
SPT::*AcΔNaeI*-A8	T_5	3	1	33
SPT::*AcΔNaeI*-B3	T_7	16	4	25
SPT::*AcΔNaeI*-B10	$T_6 + T_7$	29	3	10
sAcΩ	T_3	3	3	100
Hm^R Ds	T_3	78	14	18
FCA transgenes	T_3	50	5	10
Landsberg *erecta* × Columbia RI lines	F_{10}	118	2	1.7
Landsberg *erecta* plants	–[d]	123	0	<0.8
Landsberg *erecta* regenerants	R_1	65	0	<1.5

[a] Five transformants carrying *SPT*::*Ac* and *SPT*::*AcΔNaeI* constructs were analyzed. The prefixes A and B indicate the alternative orientations of the *SPT–Ac* construct relative to the *NPTII* fusion within the T-DNA: A, parallel; B, opposite.
[b] Individuals taken at the T_4, T_5, or T_6 generation (with T_1 being the primary transformant), which were identified as carrying *Ac* excisions (fully streptomycin resistant), were selfed; between one and 26 of their progeny (at T_5, T_6, or T_7) were analyzed for *Tag1* insertions. For example, from transformant *SPT*::*Ac*-A17, three streptomycin-resistant (T_6) individuals were selfed, and 19, 12, and 26 (totaling 57) progeny were analyzed.
[c] Leaf material from pooled progeny of the 57 lines was used to isolate DNA for DNA gel blot analysis. Three independent transformants carrying *sAcΩ*, 78 independent transformants carrying *Hm^R Ds* (which had not been crossed to an *Ac* transposase source), and 50 transformants carrying the *FCA* transgenes were analyzed in the T_3 generation. The 118 Landsberg *erecta* × Columbia RI lines were the fraction of the 298 RI lines that contained *Tag1*. DNA was also isolated and analyzed from 123 individual Landsberg *erecta* plants that had not been transformed or used in previous genetic analysis and from 65 independent Landsberg *erecta* regenerants.
[d] –, not applicable.

enced a transformation procedure at any stage in their history and were never intentionally exposed to *Ac* transposase (*Ac*-hybridizing sequences were shown to be absent in all of the F_{10} lines). Because *Tag1* is not present in the Columbia ecotype, only a proportion of the RI lines showed hybridization with *Tag1* sequences. Of those that did (118), one RI line showed two *Tag1* transposition events. Thus, the frequency of *Tag1* transposition in this nontransgenic population is estimated at ~2%. The significance of both *Tag1* transpositions occurring in the same line is unclear. It may indicate that once one transposition occurs, a second is more frequent, or that a rare combination of Landsberg *erecta* and Columbia alleles present in that RI line caused higher *Tag1* activity.

The two *Tag1* transpositions had occurred in RI line 25 by the bulked F_{10} generation. These events were further investigated by analyzing individual plants from the F_3 and F_{10} generations. Of the six F_3 individuals analyzed, none had unique *Tag1* reinsertions, whereas of four F_{10} individuals, one had inherited one of the new *Tag1* reinsertion events (Figure 1). Therefore, at least one of the transposition events in RI line 25 is likely to have occurred as a late event in the F_9 generation.

The RI lines provided a convenient and large nontransgenic population in which to analyze *Tag1* activity; however, we could not rule out the possibility that factors inherited from the Columbia ecotype might suppress *Tag1* activity. To confirm the low *Tag1* activity in nontransgenic lines, *Tag1* activity was analyzed by DNA gel blot analysis of an additional 123 individual Landsberg *erecta* plants. No new *Tag1* insertions were detected in this population; thus, the frequency of *Tag1* transposition was <0.8%.

The activity of *Tag1* was clearly higher in the transgenic lines analyzed compared with the nontransgenic lines, suggesting that events occurring during either Agrobacterium transformation or the regeneration process stimulate *Tag1* activity. To analyze the effect of tissue culture and regeneration on *Tag1* activity, 65 independent Landsberg *erecta* plants were regenerated from root explants in the absence of selection for transformation. DNA was isolated and analyzed, as was done for the other populations. No new *Tag1* insertions were detected, giving a frequency of *Tag1* transposition at <1.5%. Thus, steps during Agrobacterium infection, selection for transformation, or T-DNA integration are likely to induce the high activity of *Tag1*.

High *Tag1* Activity in Transgenic Lines Is Maintained in Successive Generations

The presence of different *Tag1* insertions in related progeny up to six generations after the transformation event suggested that *Tag1* activity remained high and was not just induced during transformation. To analyze this further, *Tag1* transposition was analyzed in two T_4 plants that were then

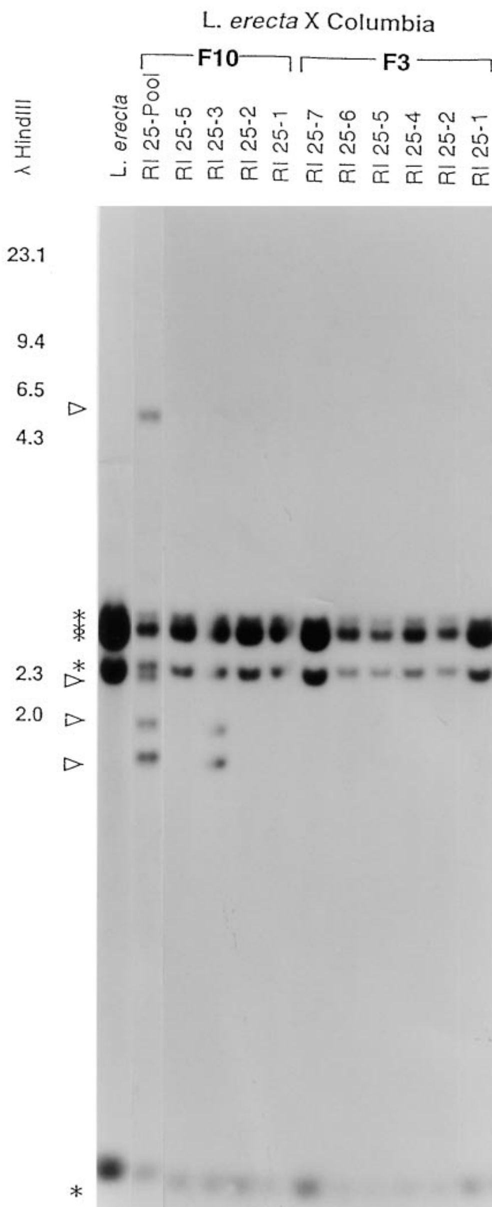

Figure 1. Analysis of *Tag1* Transposition in the Landsberg *erecta* and Columbia RI Line 25.

Genomic DNA was isolated from individual F_3 and F_{10} plants, pooled F_{10} plants, and parental Landsberg *erecta* (L. *erecta*), digested with HindIII, and used to generate a DNA gel blot. The blot was probed with the internal 1.4-kb EcoRI fragment of *Tag1*. Using this digest and probe combination, each *Tag1* transposition event would result in two novel cross-hybridizing HindIII fragments. The five HindIII fragments that cross-hybridized with *Tag1* in the progenitor are indicated by asterisks. The second and third largest fragments run as a doublet. HindIII fragments from transposed *Tag1* elements are indicated by open arrowheads. Numbers at left indicate the lengths (in kilobases) and positions of λ HindIII fragments.

crossed, and 74 of their "grandchildren" (T_6 plants) were used in DNA gel blot analysis of germinal *Tag1* insertions. The results are shown in Table 3. The T_4 parents (transformants *ΔNaeIsAc[GUS]-1* and *Hm^{R}Ds*-B1 [Bancroft et al., 1992] taken through three generations of selfing before the cross) carried a total of five *Tag1* insertions. Multiple F_1 plants were generated and selfed, and F_2 seed was collected. Plant material from bulked F_3 families was used to assess the genotype of the F_2 plants. The numbers of independent, novel *Tag1* insertions per number of F_2 plants analyzed from each F_1 generation are shown in Table 3. The frequency of new insertions per F_2 plant varied from 0 to 150%, with the 150% resulting from one of the two F_2 plants analyzed carrying two new insertions. Overall, there were 23 independent insertions in 74 F_2 plants that were not present in the T_4 parents. These transposition events will have occurred in the T_5 generation and been germinally transmitted to the T_6 progeny. Thus, we can estimate the frequency of *Tag1* transpositions in the T_5 generation to be 23 of 74, or 31%.

Table 3. *Tag1* Activity in the T_5 Generation

F_1 Plant[a]	No. of F_2 Individuals Analyzed	No. of Novel *Tag1* Insertions[b]
st8	8	3
gp17	8	2
gp5	7	0
gp18	7	1
gp12	5	0
st1	5	3
sb3	4	2
gp11	4	2
gt19	3	1
sp4	3	0
gb12	2	1
sb5	2	1
gh22	2	3
gp14	2	1
gt18	2	1
gb9	2	0
gh9	2	1
sp8	2	0
st7	2	0
st10	2	1

[a] F_1 plants were from a cross between two T_4 plants carrying an *Ac* transposase source and a *Ds* element (*ΔNaeIsAc[GUS]-1* and *Hm^{R}Ds-B1*, respectively; Bancroft et al., 1992). Fully streptomycin-resistant (resulting from a germinal *Ds* excision) F_2 seedlings were selected from 20 F_1 plants.

[b] Plant material from bulked F_3 families was used to assess the genotype of the F_2 plants. The number of independent, novel *Tag1* insertions per number of F_2 plants analyzed from each F_1 generation is shown.

Map Position of *Tag1* Elements

The Landsberg *erecta* genome carries two copies of *Tag1* (Frank et al., 1997). Because *Tag1* was absent from the Columbia ecotype, the elements could be mapped using the Landsberg *erecta*/Columbia RI lines. Apart from new *Tag1* transpositions in RI line 25 (described above), only two other RI lines showed a pattern that differed from the Landsberg *erecta* parent. RI line 365 lacked two and RI line 398 lacked one of the HindIII restriction fragments that hybridized with *Tag1.* Neither of these lines showed new *Tag1* reinsertion events, so these events were not scored as transpositions. It is difficult to distinguish *Tag1* excisions not accompanied by reinsertions from recombination events. However, we can conclude that the two *Tag1* elements cosegregated in 116 (of the 118 showing any hybridization with *Tag1*) RI families, indicating that they are tightly linked in the *A. thaliana* genome. The *Tag1* elements mapped to the lower arm of chromosome 1 (Figure 2). The *CHL1* locus that was mutated by a *Tag1* insertion is on the upper arm of chromosome 1, ~90 centimorgans away from the donor site (Tsay et al., 1993b).

Distribution of *Tag1* Sequences in Different *A. thaliana* Ecotypes and *Arabidopsis* Species

In a previous study (Tsay et al., 1993b), it was reported that *Tag1* sequences were present in *A. thaliana* ecotype Landsberg *erecta* but absent in the ecotypes Columbia and Ws. We have extended this study and looked at a large number of *A. thaliana* ecotypes and species for sequences with homology to *Tag1.* We have included ecotypes C24 and Nossen-0 (No-0), which have been extensively used for T-DNA and transposon-tagging experiments (Koncz et al., 1989; Fedoroff and Smith, 1993). *Tag1* sequences were present in Landsberg *erecta*, S96, Dijon-G, Graz, and No-0 (Figure 3). Dijon-G carries the same *Tag1* fragments as Landsberg *erecta* carries. S96 shows three of the five fragments present in Landsberg *erecta*, whereas Graz and No-0 have two hybridizing fragments; one of these (the internal BglII fragment) is common to those present in Landsberg *erecta.* These ecotypes therefore appear to contain one copy of a *Tag1* element. A different accession of No-0 has been found to carry no *Tag1* hybridizing fragments (N. Crawford, unpublished results). *Tag1* cross-hybridizing fragments were not present in the following *A. thaliana* ecotypes: Warschau-1, C24, Allerup-0, Stockholm, Enkheim, Osthammar, Bensheim, Estland-0, Wilna/Litauen, Kopenhagen, Niederzenz, RLD, Tenela, and Cape Verde Islands (data not shown for all ecotypes). A screen of an additional 35 ecotypes has shown *Tag1* elements present in 16 of them (M. Frank and N. Crawford, unpublished results).

The four *Arabidopsis* species tested were *A. wallichii*, *A. griffithiana*, *A. korshinskyi*, and *A. suecica.* They all had numerous *Tag1* cross-hybridizing fragments, indicating that they have multiple *Tag1* elements (Figure 3). The BglII enzyme used in this analysis yields both internal and flanking fragments when hybridized with the internal EcoRI fragment of *Tag1.* We interpret the large number of fainter fragments in the *A. griffithiana* and *A. suecica* lanes to represent different flanking fragments. Further analysis of *A. wallichii* and *A. griffithiana* DNA digested with EcoRI (which should yield only internal fragments) confirmed this interpretation because only two and three EcoRI fragments hybridized, respectively, with the internal 1.4-kb EcoRI fragment, compared with the two found in Landsberg *erecta* (data not shown). The faint hybridization signal of some of the fragments in *A. griffithiana* and *A. suecica* may reflect sequence divergence of different members within this transposon family. The restriction fragments hybridizing with *Tag1* were similar in *A.*

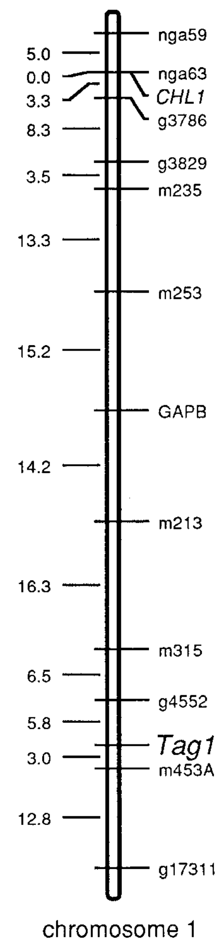

Figure 2. Genomic Location of *Tag1* Elements.

Tag1 elements cosegregated in 116 of 118 of the Landsberg *erecta* and Columbia RI lines and were linked to markers mapping to the lower half of chromosome 1. The position of *CHL1*, the locus initially used to identify *Tag1*, is also shown. Interval lengths in centimorgans are shown at left.

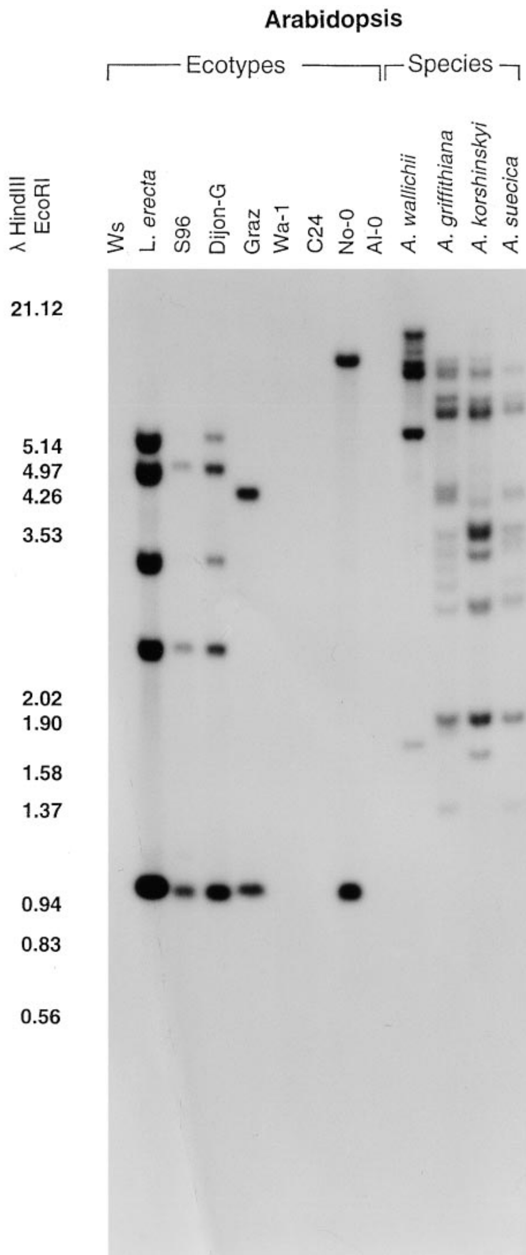

Figure 3. Distribution of *Tag1*-Related Sequences in Different *A. thaliana* Ecotypes and *Arabidopsis* Species.

A DNA gel blot of BglII-digested genomic DNA from different *Arabidopsis* ecotypes and species was probed with the internal EcoRI fragment of *Tag1*. The stronger hybridization of *Tag1* sequences with fragments in Landsberg *erecta* (L. *erecta*) compared with the other *A. thaliana* ecotypes reflects slightly higher levels of DNA in that lane. Numbers at left indicate the lengths (in kilobases) and positions of λ HindIII and EcoRI fragments. Wa-1, Warschau-1; Al-0, Allerup-0.

griffithiana and *A. suecica*, but these differed from other species, including *A. thaliana.* The presence of multiple *Tag1* elements in the *Arabidopsis* species and the low copy number or absence from most of the *A. thaliana* ecotypes suggest that *Tag1* elements have been lost from the genomes of most of the *A. thaliana* ecotypes, perhaps through a low frequency of reinsertion after excision.

DISCUSSION

Tag1 was first identified as an active transposable element in a population of transgenic *A. thaliana* (Landsberg *erecta*) plants containing a maize *Ac* element. The frequency of excision of *Tag1* from the *CHL1* locus (where it was first detected) was extremely high, with 30% of the chlorate-resistant individuals reverting to chlorate sensitivity. The goal of this study was to investigate the basis for the high activity of *Tag1*.

Tag1 activity was similar in a large number of transgenic lines derived from 136 independent transformants carrying either active *Ac* or *sAc* elements (expressing *Ac* transposase), *Ds* elements (not expressing *Ac* transposase), or *FCA* transgenes. This showed that the high activity of *Tag1* was not caused by the presence of *Ac* transposase or any sequences associated with *Ac* or *Ds* elements. However, the frequency of *Tag1* transposition was five- to 125-fold higher in transgenic lines compared with nontransgenic plants. All of the Landsberg *erecta* transgenic lines had been through a procedure involving Agrobacterium transformation of root explants and kanamycin selection. The T_2 seeds from the selfed T_1 primary transformants were then germinated on kanamycin-containing medium, and seedlings were grown on this medium until large enough to be transplanted to soil. To begin to address at which stage of this procedure *Tag1* was activated, we analyzed the frequency of *Tag1* transposition in Landsberg *erecta* plants that had been regenerated from root explants but that had not experienced Agrobacterium or selection for transformation. *Tag1* activity was $<1.5\%$ in these plants, so the increased frequency of *Tag1* transposition appeared to be the result of either Agrobacterium transformation and T-DNA integration or antibiotic selection. Once activated, the *Tag1* elements continued to transpose through successive generations, at least until the T_6 generation.

Transposable elements have been shown to be activated in maize and tobacco by tissue culture, plant disease, UV light, and γ irradiation (Dellaporta et al., 1984; Peschke et al., 1987; Walbot, 1988, 1992; Peschke and Phillips, 1991; Hirochika, 1993). It is postulated that these stresses activate transposable elements, thus generating allelic variation that may help the organism in new situations. The activation of *Tag1* in transgenic *A. thaliana* lines appears to represent another example of this type of activation. It is not difficult to envisage how Agrobacterium transformation and T-DNA integration might activate stress responses, which would lead to transposable element activation.

Tag1 elements were tightly linked and mapped to the bottom of chromosome 1. The reported mutation caused by *Tag1* is at the *CHL1* locus, which is on the top arm of chromosome 1, ~90 centimorgans from the donor site. Many more transposition events will have to be analyzed before it becomes clear whether *Tag1* shows a tendency to transpose to linked sites in the genome. Analysis of the pattern of *Tag1* transposition and the factors that affect *Tag1* transposition should help in optimizing strategies that use *Tag1* elements as effective insertional mutagens in Landsberg *erecta.*

The absence of *Tag1* elements in ecotypes C24 and Ws means that nontagged mutants from T-DNA tagging experiments in these ecotypes cannot be attributed to activity of *Tag1* elements. A number of mutations identified in Landsberg *erecta* populations that contained transposed *Ac* or *Ds* elements but that were not *Ac*- or *Ds*-tagged were analyzed to determine whether they were tagged with *Tag1* elements. None of the five mutants analyzed cosegregated with *Tag1* elements (A. Bhatt and C. Dean, unpublished results). It is possible that other endogenous transposons or retrotransposons could account for the background mutations in such experiments.

Analysis of the hybridization pattern of *Tag1* elements contributes to our understanding of the relationship of the *A. thaliana* ecotypes and *Arabidopsis* species. The identical hybridization pattern of *Tag1* elements in Landsberg *erecta* and Dijon-G suggests that these ecotypes may have a common origin. Based on a study of chloroplast DNA restriction sites, Price et al. (1994) suggest that *A. suecica* is more closely related to *A. thaliana* than to *A. griffithiana* or *A. wallichii.* However, the pattern of restriction fragments hybridizing with *Tag1* suggests a different relationship, with *A. suecica* and *A. griffithiana* being more closely related to each other than to *A. thaliana.* Why such a high proportion of *A. thaliana* ecotypes lacks *Tag1* sequences is not clear. It may be due to the loss of the elements, perhaps by excision events not associated with reinsertion. This may be the explanation for why different No-0 accessions carry different *Tag1* complements. The study of *Tag1* and related elements will reveal much more about the different *Arabidopsis* species and ecotypes and will enable *Tag1* to be exploited more widely as an insertional mutagen.

METHODS

Plant Lines

The transgenic lines used in the study have been described elsewhere. All of the plants were derived from *Agrobacterium tumefaciens* transformation of root tissue (Valvekens et al., 1988) of *Arabidopsis thaliana* ecotype Landsberg *erecta.* The lines containing an active, mobile *Activator* (*Ac*) element carried either a wild-type autonomous *Ac* cloned into the streptomycin resistance gene (Dean et al., 1992) or a modified autonomous *Ac* element, termed *AcΔNaeI*, in which 537 bp (between NaeI restriction sites) had been deleted from the 5′ untranslated leader of the *Ac* transposase gene. This deletion resulted in higher somatic and germinal excision of the *Ac* element in *A. thaliana* (Lawson et al., 1994). The lines containing a stabilized *Ac* element (*sAc*), which still had an active *Ac* transposase source, carried either *ΔNaeIsAc* (Bancroft et al., 1992) or an *sAcΩ* element in which 82 bp of the 5′ untranslated sequence of the tobacco mosaic virus coat protein (termed Ω sequence) had been cloned into the NaeI site of *ΔNaeIsAc*. The lines containing a nonautonomous *Dissociation* (*Ds*) element carried an Hm^R*Ds* element generated by replacement of an internal fragment of the *Ac* transposase gene with a 35S–hygromycin marker (Bancroft et al., 1992). The lines containing the *FCA* transgenes carried the cauliflower mosaic virus 35S promoter fused to *FCA* transcripts β or δ, the *FCA* promoter fused to *FCA* transcript γ, or the *FCA* promoter fused to β-glucuronidase (Macknight et al., 1997; R. Macknight and C. Dean, unpublished results).

The nontransgenic plants were either independent Landsberg *erecta* plants, F_{10} populations of the recombinant inbred (RI) lines derived from a cross between Landsberg *erecta* and Columbia ecotypes (Lister and Dean, 1993), or Landsberg *erecta* regenerants generated using the Valvekens et al. (1988) procedure but transferred to shoot-inducing medium (Valvekens et al., 1988) lacking kanamycin. They were grown until just before they flowered.

Preparation and Analysis of Plant Genomic DNA

High molecular weight plant genomic DNA was prepared from either pooled or individual plants by using a hexadecyltrimethyl-ammonium bromide—based method (Dean et al., 1992). Approximately 1 μg of genomic DNA was cut with the appropriate restriction enzyme, electrophoresed on a 0.8% agarose gel, and subjected to DNA gel blot analysis. The filters were probed with the 1.4-kb internal EcoRI fragment of *Tag1* (Tsay et al., 1993b) at 65°C and washed with prewarmed 2 × SSC (1 × SSC is 0.15 M NaCl and 0.015 M sodium citrate) and 0.1% SDS at 65°C for 15 min and 0.5 × SSC and 0.1% SDS at 65°C for 15 min, and then exposed to x-ray film at −70°C.

Mapping of *Tag1* Elements

The internal EcoRI fragment of *Tag1* was used to probe DNA gel blot filters of HindIII-digested genomic DNA from 298 F_{10} RI families (Lister and Dean, 1993). The segregation data were compared with those of 379 other loci by using the Mapmaker program of Lander et al. (1987).

Source of Ecotypes and Species

The different *Arabidopsis* ecotypes and species were acquired from the AIS collection held by A.R. Kranz (Fachbereich Biologie Universität Frankfurt/Main, Germany) and are now at the Nottingham Stock Centre (Nottingham, UK).

ACKNOWLEDGMENTS

We thank Dr. Melanie Stammers for providing the gel blots and doing the hybridization of *Tag1* with 298 RI lines and Dr. Jonathan Clarke for providing DNA from the different *Arabidopsis* ecotypes and species. We also thank Drs. Patti Springer, Rob Martienssen, and Venkatesan Sundaresan for providing DNA of transformants carrying *Ds*; June Swinburne for providing the *sAcΩ* transformant; and Paul Jarvis for critical reading of the manuscript. This work was supported

by Biotechnology and Biological Sciences Research Council Grant No. PG208/0609 under the authority given by the Ministry of Agriculture, Food and Fisheries license PHF1418A/952/34.

Received July 14, 1997; accepted December 26, 1997.

REFERENCES

Aida, M., Ishida, T., Fukaki, H., Fujisawa, H., and Tasaka, M. (1997). Genes involved in organ separation in Arabidopsis: An analysis of the *cup-shaped cotyledon* mutant. Plant Cell **9,** 841–857.

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

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

Dellaporta, S.L., Chomet, P.S., Mottinger, J.P., Wood, J.A., Yu, S.-M., and Hicks, J.B. (1984). Endogenous transposable elements associated with virus infection in maize. Cold Spring Harbor Symp. Quant. Biol. **49,** 321–328.

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

Fedoroff, N.V., Wessler, S., and Shure, M. (1983). Isolation of the transposable maize controlling elements *Ac* and *Ds.* Cell **35,** 235–242.

Frank, M.J., Liu, D., Tsay, Y.-F., Ustach, C., and Crawford, N. (1997). *Tag1* is an autonomous transposable element that shows somatic excision in both Arabidopsis and tobacco. Plant Cell **9,** 1745–1756.

Gallie, D.R., Sleat, D.E., Watts, J.W., Turner, P.C., and Wilson, T.M.A. (1987). The 5′-leader sequence of tobacco mosaic virus RNA enhances the expression of foreign gene transcripts *in vivo* and *in vitro.* Nucleic Acids Res. **15,** 3257–3273.

Hirochika, H. (1993). Activation of tobacco retrotransposons during tissue culture. EMBO J. **12,** 2521–2528.

Koncz, C., Martini, N., Mayerhofer, R., KonczKalman, Z., Koerber, H., Redei, G.P., and Schell, J. (1989). High-frequency T-DNA–mediated gene tagging in plants. Proc. Natl. Acad. Sci. USA **86,** 8467–8471.

Konieczny, A., Voytas, D.F., Cummings, M.P., and Ausubel, F.M. (1991). A superfamily of *Arabidopsis thaliana* retrotransposons. Genetics **127,** 801–809.

Lander, E.S., Green, P., Abrahamson, J., Barlow, A., Day, M.J., Lincoln, S.E., and Newberg, L. (1987). Mapmaker: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics **1,** 174–181.

Lawson, E., Scofield, S., Sjodin, C., Jones, J., and Dean, C. (1994). Modification of the 5′ untranslated leader region of the maize *Activator* element leads to increased activity in *Arabidopsis.* Mol. Gen. Genet. **245,** 608–615.

Lister, C., and Dean, C. (1993). Recombinant inbred lines for mapping RFLP and phenotypic markers in *Arabidopsis thaliana.* Plant J. **4,** 745–750.

Macknight, R., Bancroft, I., Page, T., Lister, C., Schmidt, R., Love, K., Westphal, L., Murphy, G., Sherson, S., Cobbett, C., and Dean, C. (1997). *FCA*, a gene controlling flowering time in Arabidopsis, encodes a protein containing RNA-binding domains. Cell **89,** 737–745.

Peleman, J., Cottyn, B., Van Camp, W., Van Montagu, M., and Inzé, D. (1991). Transient occurrence of extrachromosomal DNA of an *Arabidopsis thaliana* transposon-like element, *Tat1.* Proc. Natl. Acad. Sci. USA **88,** 3618–3622.

Pelissier, T., Tutois, S., Deragon, J.M., Tourmente, S., Genestier, S., and Picard, G. (1995). *Athila*, a new retroelement from *Arabidopsis thaliana.* Plant Mol. Biol. **29,** 441–452.

Peschke, V.M., and Phillips, R.L. (1991). Activation of the maize transposable element *Suppressor-mutator* (*Spm*) in tissue culture. Theor. Appl. Genet. **81,** 90–97.

Peschke, V.M., Phillips, R.L., and Genenbach, B.G. (1987). Discovery of transposable element activity among progeny of tissue-culture derived maize plants. Science **238,** 804–807.

Price, R.A., Palmer, J.D., and Al-Shehbaz, I.A. (1994). Systematic relationships of *Arabidopsis*: A molecular and morphological perspective. In *Arabidopsis*, E.M. Meyerowitz and C.R. Somerville, eds (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press), pp. 7–19.

Sundaresan, V., Springer, P., Volpe, T., Haward, S., Jones, J., Dean, C., Ma, H., and Martienssen, R. (1995). Patterns of gene action in plant development revealed by enhancer trap and gene trap transposable elements. Genes Dev. **9,** 1797–1810.

Swinburne, J., Balcells, L., Scofield, S.R., Jones, J.D.G., and Coupland, G. (1992). Elevated levels of *Activator* transposase mRNA are associated with high frequencies of *Dissociation* excision in Arabidopsis. Plant Cell **4,** 583–595.

Tsay, Y.-F., Schroeder, J.I., Feldmann, K.A., and Crawford, N.M. (1993a). The herbicide sensitivity gene *CHL1* of Arabidopsis encodes a nitrate-inducible nitrate transporter. Cell **72,** 705–713.

Tsay, Y.-F., Frank, M.J., Page, T., Dean, C., and Crawford, N.M. (1993b). Identification of a mobile endogenous transposon in *Arabidopsis thaliana.* Science **260,** 342–344.

Valvekens, D., Van Montagu, M., and Van Lijsebettens, M. (1988). *Agrobacterium tumefaciens*–mediated transformation of *Arabidopsis thaliana* root explants by using kanamycin selection. Proc. Natl. Acad. Sci. USA **85,** 5536–5540.

Velten, J., Velten, L., Hain, R., and Schell, J. (1984). Isolation of a dual plant promoter fragment from the Ti plasmid of *Agrobacterium tumefaciens.* EMBO J. **3,** 2723–2730.

Voytas, D.F., and Ausubel, F.M. (1988). A *copia*-like transposable element family in *Arabidopsis thaliana.* Nature **336,** 242–244.

Voytas, D.F., Konieczny, A., Cummings, M.P., and Ausubel, F.M. (1990). The structure, distribution and evolution of the *Ta1* retrotransposable element family of *Arabidopsis thaliana.* Genetics **126,** 713–721.

Walbot, V. (1988). Reactivation of the *Mutator* transposable element system following gamma irradiation of seed. Mol. Gen. Genet. **212,** 259–264.

Walbot, V. (1992). Reactivation of *Mutator* transposable elements of maize by ultraviolet light. Mol. Gen. Genet. **234,** 353–360.

Warren, W.D., Atkinson, P.W., and O'Brochta, D.A. (1994). The *Hermes* transposable element from the house fly, *Musca domestica*, is a short inverted repeat-type element of the *hobo*, *Ac* and *Tam3* (*hAT*) element family. Genet. Res. **64,** 87–97.