Characterization of the Germinal and Somatic Activity of the Arabidopsis Transposable Element Tag1

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ABSTRACT

Tag1 is an autonomous transposon of Arabidopsis thaliana. The excision behavior of Tag1 during reproductive and vegetative development was examined using CaMV 35S-Tag1-GUS constructs. Germinal reversion frequencies varied from 0 to 27% and correlated with Tag1 copy number. Southern blot and somatic sector analyses indicated that each revertant was derived from an independent excision event, and approximately 75% of the revertants had new Tag1 insertions. Revertants were obtained with similar frequencies from the male and female parents. In flowers, small somatic sectors were observed in siliques, carpels, petals, and sepals whereas stemlike organs (filaments and pedicels) had larger sectors. No sectors encompassing entire flowers or inflorescences were observed, however, indicating that excision occurs late in flower development and rarely in inflorescence meristems. Late excision was also observed during vegetative development with 99.8% of leaves showing small sectors encompassing no more than 20 cells. Roots and cotyledons, however, showed larger sectors that included entire lateral roots and cotyledons. These results indicate that Tag1 can excise in the embryo and all the organs of the plant with the timing of excision being restricted to late stages of vegetative and reproductive development in the shoot.

An endogenous transposable element, Tag1 is found in the plant Arabidopsis thaliana. It was discovered in a chlorate resistant mutant that had an insertion in the nitrate transporter gene CHL1 (Tsay et al. 1993). The insertion (Tag1) is 3.3 kb in length, has 22-bp inverted repeats at both ends, and duplicates 8 bp of genomic DNA upon insertion. When it excises from the chl1 locus, it produces chlorate sensitive revertants leaving behind small insertions or "footprints." Tag1 is found in the Landsberg erecta ecotype of Arabidopsis but not in the Columbia or WS ecotypes. Sequence comparisons have revealed that Tag1 is a member of the Ac superfamily of transposons (also called hAT family), which include Bg from maize, Slide from tobacco, Tam3 from snapdragon, Hobo from Drosophila, and Hermes from the housefly (Calvi et al. 1991; Warren et al. 1994; Grappin et al. 1996).

The initial studies of Tag1 showed that it is active and can excise to produce germinial revertants. To better characterize the transposition behavior of Tag1, we introduced it into a 35S-GUS reporter gene (Jefferson 1989), which has been used to follow transposon excision in plants (Finnegan et al. 1989; Masson and Fedoroff 1989; Lawson et al. 1994). When 35S-Tag1-GUS constructs were transformed into tobacco, a heterologous host, Tag1 excised to produce somatic sectors in leaves (Frank et al. 1997). No excision activity was seen when a GUS construct containing a defective Tag1 element (constructed in vitro) was used. We concluded that Tag1 is an autonomous element capable of independent excision. When Tag1 was introduced into Arabidopsis ecotypes that contain no Tag1 elements, it excised to produce small somatic sectors in leaves and both small and large sectors in roots in the four to five transgenic lines examined. Germinial revertants also appeared as completely blue staining progeny.

Having established that Tag1 is an autonomous element capable of both somatic and germinial excision, we wished to determine the timing and frequency of Tag1 excision and search for any consistent pattern that would indicate some form of developmental control. Over 40 independent transgenic plants containing 35S-Tag1-GUS insertions located in different regions of the genome were generated for this study. For each line, the somatic and germinial excision behaviors of the introduced Tag1 elements were examined. Analysis of the location and size of sectors gives us an indication of the developmental timing of excision, which, when it occurs in the cell lineages that give rise to the gametes, determines the number and genetic relationship of the germinial revertants. We also examined the inheritance of revertant alleles from the male and female parents, the frequency with which Tag1 reinserts in the genome, and the pattern of Tag1 reinsertion bands in the revertant progeny. The results of these experiments indicate that Tag1 excision is restricted to late stages of shoot development and produces independent germinial revertants most...
Plant growth and histochemical staining: Arabidopsis seeds were sown on GM medium with kanamycin (30 μg/ml) as described (Valvencs et al. 1988). Plants were grown under continuous light at 23–25°C. Seven days after germination, kanamycin-resistant seedlings were either stained for GUS expression or transferred to soil for further growth. Histochemical assays for GUS expression were performed as described (Jefferson 1987). Samples of plant tissue were directly submerged into solution containing 50% formamide, 5× Denhardt’s, 0.5% SDS, 100 μg/ml salmon sperm DNA, and 6× SSPE at 42°C for 4 hr. After prehybridization, 20–40 ng 32P-labeled DNA was added, and membranes were hybridized at same condition for 24 hr. After hybridization, membranes were washed in 2× SSPE, 0.5% SDS two times, 0.1× SSPE, and 0.1% SDS two times. The first three washes were carried out at room temperature, each for 20 min; the final wash was at 42°C for 40 min.

RESULTS

Tag1 excision was monitored in transgenic plants using a GUS reporter gene as described previously (Frank et al. 1997). Briefly, Tag1 was inserted between the CaMV 35S promoter and the GUS coding region. Tag1 blocks GUS gene expression, and upon excision, GUS expression is restored. The 35S promoter is a constitutive promoter allowing us to detect Tag1 excision in virtually all tissues (Benfey et al. 1989). Cells inheriting the excision product are detected histochemically as blue-staining sectors. The number of blue sectors is an indication of Tag1 excision frequency; sector size reflects the timing of Tag1 excision with small sectors indicating events occurring late in organ development. Progeny that are germinal revertants will stain completely blue. The Arabidopsis ecotype Columbia was the host for these experiments because it contains no endogenous Tag1 elements as determined by Southern blot analysis (Tsay et al. 1993). Forty-four independent transgenic plants containing the 35S-Tag1-GUS construct were generated by Agrobacterium-mediated transformation and selected with kanamycin. Progeny from each of these plants (making up a line) were stained for GUS expression.

Tag1 germinal reversion frequency: The germinal reversion frequencies (or rate) for each of the 44 transgenic plants were determined and then compared with the number of Tag1 elements in the genome of each plant. Germinal reversion frequency is defined here as the percentage of germinal revertants (completely blue staining seedlings) among all the kanamycin-resistant progeny, i.e., those containing at least one functional transgene, from a given parental line. Tag1 copy number for each line was estimated by Southern blot analysis by counting the number of bands produced by HindIII digestion of genomic DNA probed with a Tag1 fragment (probe C in Figure 3A).

Germinal reversion frequencies were found to vary from line to line even among those lines that had the same estimated Tag1 copy number (Figure 1A). For example, the frequency varied from 1.5 to 10% for lines that have six copies of Tag1. Among all the lines, the frequency varied from 0 to 10% with lines having more copies of Tag1 tending to have higher average reversion frequencies. For example, lines with only one Tag1 element had an average reversion frequency of 0.3% (± 0.4%) with four out of eight lines producing no ger-
Arabidopsis Transposon Tag1

In contrast, the average reversion frequencies in lines with eight or nine Tag1 elements were 20-fold higher. Overall, the average germinal reversion frequency was found to increase fairly linearly with Tag1 copy number (see Figure 1A legend), indicating that the effect is additive and not synergistic so that the frequency per element is approximately the same. This hypothesis would predict that a homozygous plant should have approximately two times the reversion frequency compared with a hemizygous sibling. To test this, reversion frequencies were determined for 17 individual progeny from a hemizygous plant (designated TG-3). TG-3 has an estimated seven copies of Tag1 at a single locus (segregating kanamycin-resistant to -sensitive progeny at a ratio of 3:1) and a reversion frequency of 10%. Hemizygous offspring from TG-3 had an average reversion frequency of 11.5% (±3.5%) while homozygous offspring had 20% (±4.5%) (Figure 1B). Some of the homozygous offspring had the highest reversion frequency (over 25%) we have yet observed.

The stability of the reversion frequencies from one generation to the next was assessed next by following Tag1 reversion frequencies for five consecutive generations in two different lines. Line TG-3 began with a reversion frequency of 10% and line TG-1 with a frequency of 5%. The primary transformants for each line were both hemizygous and showed a 3:1 segregation of kanamycin-resistant progeny. Reversion frequencies were determined for only hemizygous plants for each generation. The data show (Figure 2) that the average reversion frequencies do not vary much from generation to generation and are maintained for at least five

\[\text{Figure 1.—Tag1 germinal reversion rate. (A) Percent germinal revertants are given for each transgenic line and plotted based on the estimated number of Tag1 copies in the primary hemizygous transgenic plants. Kanamycin resistant progeny (approximately 250) from each line were assayed for GUS expression. Completely blue staining seedlings were scored as germinal revertants. Average reversion rates were as follows: 1 copy (8 lines), 0.3 ± 0.4; 2 copies (7 lines), 1.5 ± 1.2; 3 copies (6 lines) 1.7 ± 0.9; 4 copies (3 lines), 2.5 ± 0.9; 5 copies (5 lines), 4.3 ± 3.8; 7 copies (6 lines), 5.1 ± 2.8; 8 copies (4 lines), 6.5 ± 2.8; and 9 copies (2 lines), 6.2 ± 0.8. (B) Percent of germinal revertants are given for 9 homozygous and 7 hemizygous progeny from selfed TG-3. Progeny plants were self-pollinated, and seeds were collected from each individual plant. Seeds were plated on nutrient agar with kanamycin, and then resistant seedlings (approximately 250) were assayed for GUS expression. Completely blue staining seedlings were scored as germinal revertants.}\]

\[\text{Figure 2.—Inheritance of germinal reversion activity. Germinal reversion rates for eight plants were determined in each of five generations and for the primary transformant (generation 1). All plants tested were hemizygous. Data are average values with standard deviation. (●), Line TG-1; (○), Line TG-3.}\]
We conclude that Tag1 reversion frequencies are relatively stable from generation to generation. We also examined the inheritance of revertant alleles from the male and the female organs of a plant. Differences in the frequency or transmission of excision events in the cell lineages that produce male or female gametes can skew the number of revertants that are inherited from the male or female parent. To test this, reciprocal crosses between untransformed, wild-type plants and transgenic plants that carried the 35S-Tag1-GUS constructs were performed. To determine the contribution of revertants from the male lineage, pollen from four plants of line TG-3 (two hemizygotes and two homozygotes) were applied to flowers of untransformed plants. Kanamycin-resistant seedlings derived from F1 hybrid seeds were stained for GUS expression. Reciprocal crosses where the female contained the 35S-Tag1-GUS construct were performed to assess the contribution from the female parent. The four transgenic plants were also self-pollinated, and their progeny was stained for GUS expression.

The data show that the germinal reversion frequencies were approximately the same for both reciprocal crosses (Table 1). Approximately the same fraction of germinal revertants were observed from crosses where the male contributed the 35S-Tag1-GUS construct and from crosses where the female contributed the 35S-Tag1-GUS construct. We conclude that there is no bias in the inheritance of the germinal revertant alleles.

Examination of the data (Table 1) shows that the germinal reversion rates in progeny produced from selfing the transgenic plants is a little higher than the sum of the rates from the pairwise crosses. Assuming no segregation distortion, the frequency of germinal revertants from selfed plants should be slightly lower than the sum of the pairwise crosses because of masking of some of the revertant alleles in homozygous progeny. Our data show the opposite; the frequency is slightly higher among the progeny of selfed plants. One explanation for this result is that some excision events are occurring in the zygote, and, when a zygote is homozygous for the Tag1 transgene, the excision rate is higher. As noted above, genetic dosage does influence the rate of germinal reversion.

Once an element excises, it can either reinsert or be lost from the genome. The value of Tag1 as an insertional mutagen depends in part on its probability of reinsertion. Previously, we showed that new insertion bands could be observed on Southern blots of DNA from chlorate-sensitive plants derived from chl1::Tag1 mutants (Tsay et al. 1993). In the present study, we examined the fraction of revertant progeny from a single transgenic plant that showed new Tag1 insertion bands. Genomic DNA was analyzed by Southern blot analysis. HindIII digestion cuts Tag1 in the 35S-Tag1-GUS construct into three fragments: a central 260-bp fragment, a 2.3-kb left fragment containing the 0.8-kb 35S promoter, and a right fragment whose size depends on the position of a HindIII site in the flanking genomic sequence (Figure 3A). If the element excises and reinserts, additional bands should be observed with distinct flanking sequences on both sides. Two probes were used for this analysis; probe A is a fragment from the left side of Tag1, and probe C is from the right side (Figure 3A).

<table>
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<th>Crosses</th>
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<th>Kan(^{S}) progeny</th>
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\(\text{a}\) Primary transformant TG-3 is T\(_1\) generation. Direct progeny plant from TG-3 is defined as T\(_2\) generation, and so on.

\(\text{b}\) Wild-type, untransformed plants.

\(\text{c}\) Hemizygous plants (T\(_2\) generation).

\(\text{d}\) Homozygous plants (T\(_3\) generation).
bands after HindIII digestion and hybridization with probe C (Figure 3B, lane 15). There was no significant hybridization to DNA from untransformed plants (data not shown). DNA from 63% of the revertant lines showed new hybridization bands (Figure 3B, lanes 1–14, and data not shown). Because some of the new bands could overlap with those present in the primary transformant, the same blot was rehybridized with probe A. With this probe, only a 2.3-kb band from the 35S-Tag1-GUS construct is expected (Figure 3A). We detected three additional bands in the TG-3 transformant indicating that the internal HindIII site flanking the 35S promoter was altered in some of the integrants (Figure 3C, lane 15). Examination of the revertant lines showed new hybridization bands in 53% of the lines (Figure 3C, lanes 1–14, and data not shown). When data from both blots were combined, new Tag1 hybridizing bands were detected in 74% of the lines, which we believe is a minimal estimate. Eleven revertant lines had one new band, twelve lines had two new bands, and one line had three new bands with an average number of new bands in each revertant line being one. In summary, three-fourths of the revertants show new hybridization bands indicating that Tag1 has a high probability of reinsertion.

Another important conclusion can be made from the Southern blot data. If excision events are occurring early during the development of a plant, large sectors will be produced that contain many identical revertants. If excision events occur late, most of the revertants will arise from independent excision events. The pattern of hybridization bands for each revertant was found to be unique (Figure 3B, and data not shown). This result indicates that each revertant is independent, arising from unique excision events, and that early excision is not occurring.

**Tag1 somatic excision in siliques and flowers:** To gain more insight into the pattern of Tag1 excision during plant development, we examined the pattern of somatic sectors in various organs of the plant. If excision is occurring late during development in the cell lineages that give rise to the gametes, we should also observe small sectors in flower organs and siliques, which arise from the same floral meristems that give rise to the gametes. Eight to ten fully developed green siliques 6–8 days after pollination, and two whole inflorescences containing 8–12 flowers at different developmental stages (from buds to fully opened flowers) were examined for each plant.

Siliques from all 44 primary transformants and 20 progeny plants from each of four individually selected lines (TG-1, TG-2, TG-3, and TG-5) were examined. The primary transformants TG-1, TG-2, TG-3, and TG-5 have an estimated 9, 1, 7, and 5 copies of the 35S-Tag1-GUS construct, respectively (Figure 3 and data not shown), and show an approximate 3:1 segregation of the kanamycin-resistant phenotype indicating a single locus for the transgene(s). Siliques from plants containing a 35S-GUS construct stained completely blue (Figure 4A), while siliques from untransformed plants showed no staining (Figure 4B). For transgenic plants containing the 35S-Tag1-GUS construct, all siliques (over 1000 examined) showed GUS sectors as small blue dots or short strips (Figure 4, C–E). Small sectors were also observed on silique pedicels (Figure 4F). In a few rare cases, the entire pedicel stained blue (Figure 4G), but the GUS staining did not extend into the silique. These results indicate that the observed sectors were because of Tag1 excisions that occurred late during carpel/silique development. Because no large sectors encompassing entire siliques were found in any of the transgenic plants, we conclude that the late timing of excision was independent of Tag1 copy number and genetic dosage.
The somatic sectors described above are in the maternal tissues of the silique. By examining the embryos/seeds within the silique, one can gain insight into the pattern of germinal excision events. Blue staining seeds indicative of germinal revertants were detected in siliques (Figure 4H). The maternal tissues surrounding these seeds, the funiculus (Figure 4I) and seed coats (Figure 4J), did not stain blue. The position of the blue staining seeds in a given silique was random. Occasionally, two revertant seeds were found adjacent to each other, but the surrounding maternal tissue showed no staining (Figure 4I). Thus, we find no evidence of sectors that encompass more than a single seed. Because we found no bias in the inheritance of germinal revertants from the male versus the female lineages, approximately half of these revertants were because of excision events in the female lineage. These results suggest that germinal excision events are occurring after ovule founder cells have separated from cells that give rise to the surrounding tissues. We have also examined anthers to check for evidence of early excision events that would give rise to large or “jackpot” sectors encompassing entire anthers. We only found a few pollen grains (less than 1%) staining blue in all anthers examined (see below).

The frequency of revertant sectors was also examined in siliques to assess the variation from line to line. Sector number varied from greater than 300 per silique in one line to less than 20 per silique in others (examples are shown in Figure 4, C–E). Within a plant, there was some variation from silique to silique, but 6–8 out of 10 siliques usually had the same variegation pattern. We examined the number of sectors on the carpels (progenitor to the silique) in a line that had many silique sectors (>300) and found that the carpels showed many fewer sectors (<30). This result further supports the finding that excision events are occurring late, in this case, after pollination.

The pattern of sectors on flower organs was exam-
in any of the 44 lines, which contained a distribution of excised sectors on the petals (Figure 5, J and K). In a few rare cases, an entire filament stained blue (data not shown), but a whole blue staining anther was never observed. Examination of the pollen in over 40 individual anthers showed that all had a very low percentage of blue staining pollen (less than 1%; data not shown). No large dark staining sectors encompassing multiple organs and not an entire flower or branch were observed in any of the 44 lines, which contained a distribution of Tag1 elements at different locations. We conclude that Tag1 excises late during flower development regardless of its copy number and genetic dosage.

When the number of sectors was examined in the transgenic lines, it was apparent that frequency of somatic sectors varied greatly from line to line. Highly variegated flowers had over 100 sectors (Figure 5, C and F). Flowers with medium variegation had between 10 and 100 sectors (Figure 5, D and G). Lightly variegated flowers had less than 10 sectors on all four sepals (Figure 5, E and H). Within a plant, the excision frequency did not vary significantly between petals and sepals within a flower, among flowers within an inflorescence, nor among inflorescences. Within a line, however, the frequency varied from plant to plant. We observed one exceptional case (Figure 4L) in which half of the progeny from TG-1 had the unusual uniform blue staining pattern, which was restricted to sepals and could be because of splicing of the Tag1 sequences from the chimeric Tag1-GUS mRNA.

**Tag1 somatic excision in vegetative organs:** The pattern of somatic sectors was also examined in leaves, cotyledons, and roots to determine if the late timing of Tag1 excision observed in flower development was also occurring in vegetative development. The third and fourth true leaves from all 44 primary transformants and whole plants from the progeny of TG-1, TG-2, TG-3, and TG-5 were stained for GUS expression. Leaves were sampled when they had just reached their fully expanded state. As positive and negative controls, leaves from transgenic plant containing a 35S-GUS construct stained completely blue (Figure 6A), and leaves from untransformed plant showed no GUS staining (Figure 6B). Leaves from transgenic plants bearing 35S-Tag1-GUS construct displayed a variable number of GUS sectors (Figure 6, C–E), but the size of GUS sectors in all leaves were similar and very small (usually encompassing less than twenty cells and sometimes only one cell). Sectors in other leaves including the cauline leaves and stems also exhibited the same small size (Figure 6, F and G and data not shown). Cross sections of leaf blades showed sectors in all major cell types, including epidermal, mesophyll, vascular, and trichome cells (data not shown). Among the more than 1000 leaves examined, only two had larger sectors. One such sector is shown in Figure 6H. This sector emerged from the leaf petiole and extended into the leaf blade comprising about one-third area of entire leaf. Similar sized sectors have been derived from X-ray irradiated seed used for fate map studies (Irish and Sussex 1992); thus, this rare Tag1-induced sector most likely arose
from an excision event in the shoot meristem. Typical small GUS sectors were also present on this exceptional leaf, and other leaves from the same plants and their progeny plants showed only the typical small sectors. These results show that Tag1 excision almost never occurs in the shoot vegetative meristem but is delayed until late in leaf development after the founder cells leave the meristem to form the leaf primordium. This behavior was found in all 44 lines and is therefore independent of copy number and genomic location.

When the number of sectors per leaf were determined for each line, line TG-1 was found to have an exceptional phenotype. Initially, the number of sectors per leaf was low (less than 50) when the leaves were still small. Then at a specific stage during leaf expansion, a burst of excision activity would occur and continue for as long as the leaves were examined. To quantify this response, the number of sectors present on true leaves 1–4 were compared to the length of the leaf for five plants sampled every day for 23 days. The expansion of leaves 1 and 2 behaved very similarly and were treated as a pair. Figure 7 shows changes in leaf length; leaves 1 and 2 emerged at day 4 after seed germination and reached the maximum length at day 9 (Figure 7A); leaves 3 and 4 appeared at day 7 and 8, and reached full expansion at day 20 (Figure 7B).

A burst of excision activity during a particular interval during leaf expansion was observed (Figure 6, I–L, Figure 7, and Figure 8). For leaves 1 and 2, the burst appeared at day 9 after seed germination when leaves just finished their expansion (Figure 7A). The burst time for leaves 3 and 4 was between days 15 and 16 during the middle of leaf expansion (Figure 7B). When whole plants were stained for GUS expression at day 28 after seed germination (Figure 8), one could observe numerous sectors in leaves 1 through 4, which had reached expansion fully. In contrast, younger leaves still in the expansion stage had very few sectors. Leaf 5 appears caught in the middle of the switch with 80% of the leaf area showing sectors while leaf 6, which is only two days delayed compared with leaf 5, showed only a few sectors. This sudden tran-
position of Tag1-excision activity from leaf 5 to leaf 6 suggests that there is a switch that turns on Tag1 excision at a particular interval during leaf expansion in this line.

Lastly, Tag1 somatic sectors were examined in cotyledons and roots in progeny of primary transformants TG-1, TG-2, TG-3, and TG-5 and compared with the sectors in leaves and flowers. Whole seedlings containing 35S-GUS control constructs stained completely blue (Figure 9A), and no GUS staining was found in the seedlings from untransformed plants (Figure 9B). Seedlings from transgenic plants carrying 35S-Tag1-GUS constructs exhibited distinct sector types in cotyledons. Lines TG-1 and TG-2 exhibited a mixture of small- to medium-sized sectors (Figure 9, C and D). In line TG-3, sectors were uniformly small consisting of only a single or few cells (Figure 9, E and F). In line TG-5, GUS sectors were medium to large and usually covering one-eighth to one-half area of a single cotyledon (Figure 9, G and H). In a few rare seedlings, two entire cotyledons stained blue indicative of an excision event in the embryo (data not shown). Roots also showed a wide distribution of sector sizes. In lines TG-2 and TG-5, sectors were very small regardless of the pattern in the cotyledons (Figure 9I). However, in lines TG-1 and TG-3, both small and large sectors were observed with some sectors covering one-fourth to one-third area of primary root indicative of excision events in the embryo or root meristem (Figure 9J and K). In about 2% of seedlings stained from line TG-1, one or two whole lateral roots stained blue (Figure 9L). In rare cases, the primary root stained blue with the shoot part showing variegation (Figure 9M). These results show that the timing of excision in roots, cotyledons, and the embryo is variable and line-dependent.

Sector number for cotyledons also showed considerable variation between and within lines. The average number of GUS sectors per cotyledon in lines TG-1, TG-2, TG-3, and TG-5 were approximately 19, 1, 39, and 2, respectively. In roots, sector numbers were low regardless of their frequency in cotyledons. In our most active line TG-1, the average sector number in the whole root system was less than six (for seedlings that were 8-days old after germination; data not shown). Many seedlings from these four lines did not show any GUS sectors in roots.

In all the transgenic lines described above, the sequences immediately adjacent to Tag1 (the 35S promoter and GUS reporter gene) and the genetic background, i.e., Columbia ecotype, were all the same. To determine if the Tag1 excision behavior that we were observing is specific to the Columbia ecotype or is dependent on the sequences immediately adjacent to the transposase source, we examined Tag1 excision in a different ecotype and in a different construct. First, the 35S-Tag1-GUS construct was transformed into Landsberg erecta plants. Eight independent transgenic lines were produced. All displayed the same pattern of sectors in leaves, roots, flowers, and siliques seen in the Columbia ecotype (data not shown). Second, a defective Tag1 element (dTag1), constructed by deleting an internal 1.4-kb EcoRI fragment, was introduced into Landsberg erecta plants in the 35S-GUS reporter construct. This dTag1 has already been shown to be mobile in Landsberg (Frank et al. 1997). In these plants, the autonomous Tag1 elements, which are providing the transposase function, are not adjacent to the 35S promoter but reside next to native sequences on chromosome 1 of the Arabidopsis genome. Five independent transgenic lines were produced and all had the same pattern of sectors observed in the Columbia lines (data not shown). These results indicate that timing of exci-
sion is not ecotype-specific nor dependent on the 35S promoter being adjacent to the transposase source.

**Discussion**

Tag1 is a transposable element whose mobility has been demonstrated in both its host Arabidopsis and in a heterologous plant, tobacco (Tsay et al. 1993; Frank et al. 1997). Compared to other plant transposons, such as Ac, Spm, and Mutator in maize and Tam3 in snapdragon, the genetic and molecular properties of Tag1 are less well characterized. The original study of Tag1 relied on an insertion allele of the CHL1 gene to monitor Tag1 excision and showed that Tag1 can excise from the chl1 locus to produce chlorate-sensitive revertants (Tsay et al. 1993). Subsequently, we demonstrated that Tag1 is an autonomous element that excises during vegetative growth to produce somatic sectors in leaves and roots using a CaMV 35S-GUS reporter construct (Frank et al. 1997). In the study reported here, we characterized the timing and frequency of Tag1 excision in both reproductive and vegetative development to determine if Tag1 excision activity is developmentally controlled and if Tag1 had properties that would make it useful for insertional mutagenesis.

After analyzing over 40 independent transgenic lines of the Columbia ecotype, we found evidence of excision activity in all organs of the plant: roots, cotyledons, stem, leaves, and flowers. Examination of the pattern of sectors revealed that the timing of excision is restricted during shoot vegetative and reproductive development but is variable during root and cotyledon development. The restriction of excision activity in the shoot also applies to the cell lineages that give rise to the gametes indicating that germinal excision events are occurring very late in flower development. Because late timing of excision in shoots was observed in all lines, which, because of their independent derivation, should have Tag1 elements located in different regions of the Arabidopsis genome (although no mapping experiments have been performed), we infer that excision timing is independent of the genomic location of the Tag1 elements.

The sector patterns in leaves and flowers suggest that Tag1 excision is rarely occurring in the shoot meristem during all three stages of development: vegetative, inflorescence, and floral. Meristematic events would be expected to give rise to large sectors encompassing major portions or entire organs and branches or stripes along the stem between nodes. Examples of sectors that arise from meristem events from X-ray-irradiated seed and transgenic plants containing a modified Ac element (with a 35S promoter driving the transposase expression in a GUS reporter gene) have been published (Furner and Pumphrey 1992; Irish and Sussex 1992; Bossinger and Smyth 1996; Goldsbrough et al. 1996). These studies show sectors that include portions or all of a leaf that runs the length of the organ, multiple organs, and entire branches. These type of sectors are rarely if ever produced by Tag1 in the 35S-GUS reporter construct. The one exception is the stem-like organs of the inflorescence (filaments and pedicels), which showed a distribution of sector sizes including those that encompassed the entire stem. The excision events that gave rise to these large sectors may have occurred in the floral meristem.

The patterns observed in leaves and flowers differed from those observed in roots and cotyledons where both early and late excision events were occurring. Sector sizes were also quite variable even among progeny from a single plant. This behavior suggests that the control of excision timing is not as strict in the embryo, root, and cotyledons as it is in the shoot. The frequency of somatic excision was also found to be quite variable. However, a correlation was found between somatic excision frequency in flowers/siliques and germinal reversion frequency (data not shown), suggesting some overlap in control mechanisms.

It is interesting to compare the excision activities of Tag1 with those of other transposable elements. The timing of Tag1 excision appears to be most similar to Mutator, which shows both germinal and somatic reversion activity late in maize development (Robertson 1981; Levy et al. 1989; Levy 1990; Benetzen et al. 1993; Lisch et al. 1995). This delay in Mutator excision during development correlates with a reduction in MURB levels, one of the Mutator-encoded proteins that is expressed less abundantly in the cells that are not actively dividing (Donlin et al. 1995). The late timing of Mutator activity is not usually affected by dosage of Mutator elements, genetic background, or relative transposition activity (reviewed in Benetzen 1996). Germinal reversion events usually produce very small clusters of revertant seeds in the ear (most often single-seed sectors), similar to the behavior of Tag1 in Arabidopsis, but the rate of germinal reversion is low (often about 1/10⁶) (Brown et al. 1989; Levy et al. 1989; Walbot 1992). Most new Mutator-induced mutations, which can occur at high frequency in Mutator lines and after meiosis in both the male and female lineages (Robertson and Stinar 1993), also are represented by small clusters of seeds (from 1-11 seeds) on the ear (Robertson 1980; Robertson 1981). A “big spot” line of maize has been identified that has an altered timing pattern for Mutator and a higher frequency of germinal reversion (Walbot 1992).

The relationship between copy number and germinal reversion rates for Tag1 most resembles that described for Ac in dicots but not in maize. In maize, the timing of Ac excision in kernel development can be delayed with increasing dosage of Ac (McCintock 1950; McCintock 1951), but this effect is dependent on the level of transposase and the dosage and composition of the transactivated element (Heinlein and Starlinger 1991; Heinlein 1996). In dicots, there is a consistent increase in germinal transposition with increasing Ac.
copy number (Jones et al. 1989; Hehl and Baker 1990; Keller et al. 1993). Very high levels of transposase expression, however, have been found to inhibit Ac transposition, perhaps because of the aggregation of the transposase (Scofield et al. 1993; Heinlein et al. 1994).

The inheritance of Tag1 revertant alleles from the male and the female parents appears to be the same; therefore, approximately half of the germinal revertants in a silique arose from excision events that occurred in the female parent and half in the male. In maize, Mutator activity is inherited more efficiently through the female than the male (Walbot 1986; Bennetzen 1987; Lisch et al. 1995). For Ac at the R-n locus, the frequency of reversion was 2–3 times higher when the male (as compared with the female) contributed the Ac element (Brink and Williams 1973).

These and many other studies (reviewed in Fedoroff 1989; Fedoroff and Bossen 1992; Saedler and Gierl 1996) have shown that transposition can be regulated in plants. Tag1 excision activity also displays developmental regulation in Arabidopsis. This regulation is most pronounced in the shoot and generates independent germinal revertants.

Transposable elements have been very useful for isolating genes based solely on mutant phenotypes and for enhancer and gene trapping (reviewed in Walbot 1992; Coupland 1994; Osborne and Baker 1995; Sundaresan et al. 1995). They offer an alternative with both advantages and disadvantages to the use of T-DNA from Agrobacterium as an insertional mutagen. Gene tagging systems have been established in Arabidopsis using the maize elements Ac, Spm, and their derivatives Ds and dSpm. One may ask if Tag1 may also serve as an useful insertionional mutagen for tagging genes. Our results indicate that transgenic plants carrying a single copy of Tag1 display low-germinal excision rates, generally lower than 1%. However, for plants that have multiple copies of Tag1, the rate is much higher. The highest rate we have observed is 26% for homozygous progeny of TG-3, which have seven copies of Tag1 in the hemizygous state. This rate is encouraging for mutagenesis. We have also found that new insertion bands were observed in approximately three-fourths of the plants that had undergone a Tag1 excision event. In 47 germinal revertants from a single plant, we found no reinsertion events that gave the identical Southern blot pattern, indicating that Tag1 excision is occurring late enough to produce independent revertants. These results suggest that Tag1 will be useful for insertional mutagenesis. At the very least, Tag1 should be used as a probe to check new unstable mutations in transgenic lines that contain Tag1. The Ac-containing lines of Landsberg erecta have so far produced three tagged mutants: the original chl1::Tag1 mutant (Tsai et al. 1993), a cup-shaped cotyledon mutant (Aida et al. 1997) and a pinhead meristem-defective mutant (Lynn et al. 1997), all having Tag1 insertions that allowed cloning of the corresponding gene.

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