Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 3618–3622, May 1991
Botany

Transient occurrence of extrachromosomal DNA of an *Arabidopsis thaliana* transposon-like element, *Tat1*

(plant/*S*-adenosylmethionine/transposable element)

J. PELEMAN*, B. COTTYN, W. VAN CAMP, M. VAN MONTAGU†, AND D. INZÉ

Laboratorium voor Genetica, Rijksuniversiteit Gent, B-9000 Gent, Belgium

Contributed by M. Van Montagu, January 2, 1991

ABSTRACT **Analysis of 11 genomic clones containing the *S*-adenosylmethionine synthetase 1 gene (*sam1*) of *Arabidopsis thaliana* revealed the presence of a 431-base-pair (bp) insertion in the 3′ end of *sam1* in one of these clones. The inserted sequence, called *Tat1*, shows structural features of a transposon. It is flanked by a 5-bp duplication of the target site DNA and has 13-bp inverted repeats at its termini. Two highly homologous elements situated in a different genomic context were isolated from a genomic library. Genomic Southern analysis indicates that there are at least four copies of *Tat1* present in the *A. thaliana* ecotype Columbia genome. Different hybridization patterns are observed with DNAs derived from different ecotypes of *Arabidopsis thaliana*, indicating that the element has moved since the divergence of these ecotypes. In two populations of *A. thaliana*, linear extrachromosomal *Tat1*-homologous DNA has been observed. The presented data are consistent with the hypothesis that *Tat1* is an active transposable element.**

Transposable elements and transposon-like elements have been isolated from an increasing number of plant species over the last decade. These elements are characterized by the presence of terminally inverted repeats and the apparent ability to generate duplications of the target site upon integration (for reviews, see refs. 1 and 2). Many elements isolated from several plant species contain homologous inverted repeats. On this basis the elements *Tam1* and *Tam2* from *Antirrhinum majus* (3), *Tgm1* from soybean (4), *Spm18* from maize (5), and *Pis1* from pea (6) belong to one class of transposons starting with the sequence 5′ CACTA-- 3′, whereas *Ac/Ds* from maize (7), *Tam3* from *Antirrhinum majus* (8), *Tpc1* from *Petroselium crispum* (9), *Ips-r* from *Pisum sativum* (10), and *Tat1* from *Arabidopsis thaliana* (described in this paper) constitute a second class of transposons showing mutual homology in their inverted repeats.

This paper describes three copies of an *Arabidopsis* transposon-like element, designated *Tat1*.‡ Evidence is presented that *Tat1* is an active (nonautonomous) transposon.

MATERIALS AND METHODS

Plant Material. *A. thaliana* seeds were obtained from the Max-Planck-Institut für Züchtungsforschung (Köln, F.R.G.) (collection number K85), from M. Jacobs (Vrije Universiteit Brussel, Brussels) (collection number C24), from G. Rédei (University of Missouri) (ecotype Columbia), from K. Feldmann (DuPont) (ecotype Wassilewskija), and from M. Koornneef (Wageningen, The Netherlands) (ecotype Landsberg *erecta*).

Subclones and Preparation of DNA and Riboprobes. From the original genomic phage clone λSAM3 containing the *sam1* gene with a *Tat1* insertion in its 3′ end, a 0.7-kilobase (kb) *Pst* I–*Hin*dIII fragment containing the *Tat1* insertion was cloned in pGEM-2 yielding plasmid pTAT1A-2. From λ phage clones λTATb and λTATc, a 0.5-kb *Bam*HI–*Xho* I fragment and a 4.0-kb *Eco*RI fragment, respectively, containing the *Tat1b* and *Tat1c* isolate, were subcloned into pGEM-2 yielding plasmids pTAT1B-2 and pTAT1C-1.

For the preparation of the different DNA probes, the DNA fragments were eluted from agarose gels, and probes were prepared by using the multiprime DNA-labeling system from Amersham.

Riboprobes were prepared according to the Promega protocol using T7 polymerase.

Elution of DNA Fragments from Agarose Gels. After electrophoresis in gels prepared with ultrapure agarose (BRL), the DNA fragment was cut out. The agarose piece was pushed through a Millex-HV 0.45-μm, 13-mm filter unit (Millipore) by using a 2-ml syringe. The filter was washed once with 200 μl of water. After extraction with phenol, the fragment was precipitated with ethanol and dissolved in water, and the concentration of the fragment was estimated on agarose gel.

Screening of a Genomic Library. The genomic library used for screening has been described (11, 12). The library was screened by plaque hybridization by the procedure of Maniatis *et al.* (13) with Riboprobes transcribed from the 0.7-kb *Pst* I–*Hin*dIII fragment of pTAT1A-2. Prehybridization and hybridization were carried out as described by Peleman *et al.* (14).

Preparation of DNA. *Arabidopsis*, *Brassica*, *Nicotiana*, and *Oryza* DNA were isolated by the procedure of Dellaporta *et al.* (15).

Southern Blots. Genomic Southern hybridizations were carried out as described by Peleman *et al.* (11).

Miscellaneous Techniques. All recombinant DNA techniques not described above were as described by Maniatis *et al.* (13). The DNA sequence was determined on both strands by the procedure of Maxam and Gilbert (16). Nucleotide comparison analysis was done with the IntelliGenetics software package for molecular biologists.

RESULTS

Isolation and Sequence Analysis of an Insertion Sequence in the 3′ End of the *sam1* Gene. Eleven genomic clones containing the *S*-adenosylmethionine synthetase (*sam1*) gene were isolated from a Charon 35 library of *Arabidopsis* by using the 0.78-kb *Bgl* II–*Sal* I fragment of the *sam1* gene as a probe (11).

Restriction and hybridization analysis showed that the *sam1* gene in λ phage clone λSAM3 was present within a 4.5-kb *Eco*RI fragment instead of a 4.0-kb *Eco*RI fragment as

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

*Present address: Keygene N. V., Keyenbergseweg 6, Postbus 216, NL-6700 AE Wageningen, The Netherlands.
†To whom reprint requests should be addressed.
‡The sequences reported in this paper have been deposited in the GenBank data base [accession nos. M55077 (3′ *sam1*), M55144 (*Tat1a* isolate), M55145 (*Tat1b* isolate), and M55146 (*Tat1c* isolate)].

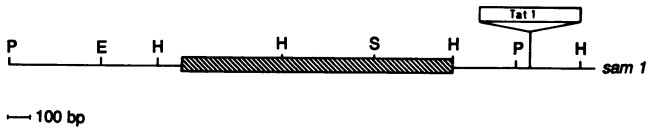

FIG. 1. Restriction map of the *saml* gene and indication of the insertion position of *Tatl* in the genomic phage clone λSAM3 (see text). The hatched box indicates the coding region of *saml*. E, *Eco*RI; H, *Hin*dIII; P, *Pst* I; S, *Sal* I.

in the 10 other *saml*-containing clones (data not shown). Detailed restriction analysis of the 4.5-kb *Eco*RI fragment and comparison with the restriction map of the original *saml* gene revealed that the λSAM3 clone contained an insertion of ≈400 bp in the 3′ end of the *saml* gene (Fig. 1). Sequence analysis of the 0.7-kb *Pst* I–*Hin*dIII fragment containing this insertion shows that a 431-base-pair (bp) sequence element is inserted 330 bp downstream from the stop codon of the *saml* gene (Fig. 2). This insertion, called *Tatl*, shows the structural characteristics of transposable elements. It is flanked by a 5-bp duplication of the target site DNA: ACGAT. The ends of the element contain a perfect 13-bp or an imperfect 16-bp inverted repeat of the sequence 5′-TGTGGATGTCG-GAXTG-3′. The inverted repeat shows homology with the terminally inverted repeat sequences of the *Ac/Ds* elements in *Zea mays* (7), the *Tam3* element of *An. majus* (8), the *Tpc1* element of *Pe. crispum* (9), and the *Ips-r* element of *Pi. sativum* (10) (Fig. 3). The element contains internally a perfect 20-bp palindrome 65 nucleotides from the left border of the element (Fig. 2).

Sequences flanking the *Tatl* element are 100% identical with the 3′ end sequences of *saml* isolates lacking the *Tatl* insertion. This observation suggests that the *Tatl* insertion has occurred recently.

Isolation of Two Additional *Tatl* Copies. Hybridization of *Arabidopsis* DNA digests with the *Tatl* element as a probe reveals several hybridizing bands (see below). To isolate additional sequences homologous to the *Tatl* element out of the *Arabidopsis* genome, the genomic Charon 35 library was screened with the 0.7-kb *Pst* I–*Hin*dIII fragment containing the *Tatl* element as a probe. For convenience, the *Tatl* insertion in the 3′ end of the *saml* gene will be referred to further as the *Tatla* isolate. Twenty-five clones hybridizing with *Tatla* were isolated for further analysis. Restriction analysis and subsequent hybridization with the *Tatla* isolate as a probe revealed two phage clones, λTATb and λTATc, containing *Tatl*-homologous DNA situated in a different genomic context than the *Tatla* isolate. These isolates will be further referred to as *Tatlb* and *Tatlc*, respectively. Hybridization analysis revealed that the region homologous to the *Tatla* element was confined to a 0.5-kb *Bam*HI–*Xho* I fragment for the *Tatlb* isolate and a 0.95-kb *Bgl* II–*Pvu* II fragment for the *Tatlc* isolate. These fragments were used for sequence analysis. The sequences of *Tatlb* and *Tatlc* are compared with the *Tatla* sequence in Fig. 2. The *Tatlb* isolate is 430 bp long and shows 92.5% and 96.0% identity

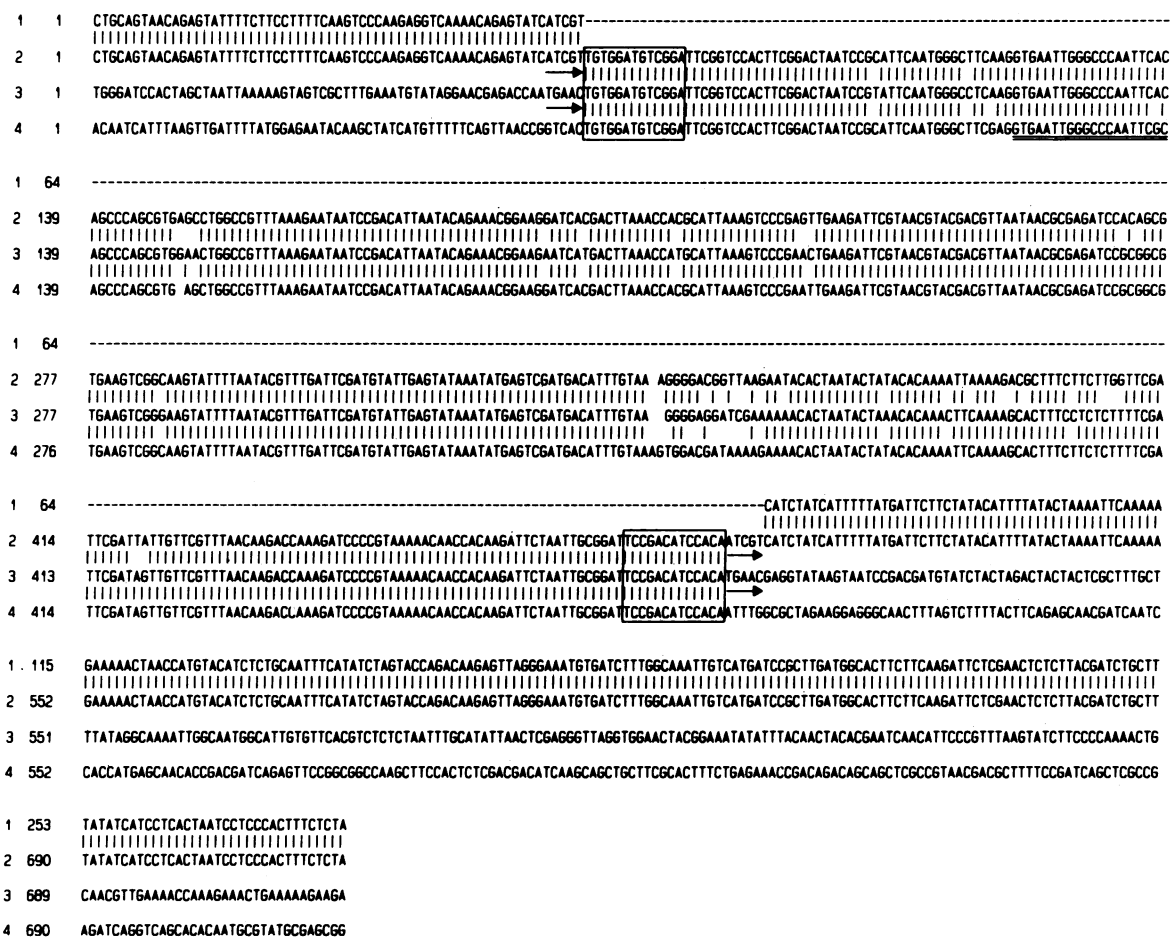

FIG. 2. Comparison of the sequences of the *Tatla*, *Tatlb*, and *Tatlc* isolates. Lines: 1, sequence of the *Pst* I–*Hin*dIII fragment in the 3′ end of *saml*; 2, sequence of the *Pst* I–*Hin*dIII fragment containing the *Tatla* insertion in the 3′ end of *saml* of phage clone λSAM3; 3, sequence of the 0.5-kb *Bam*HI–*Xho* I fragment of pTAT1B-2 containing the *Tatlb* isolate; 4, sequence of a 0.5-kb fragment of pTAT1C-1 containing the *Tatlc* isolate. Nucleotide homologies between the different sequences are indicated with vertical lines. Target site duplications are underlined with an arrow. The 13-bp inverted repeats of the *Tatl* elements are boxed. The 20-bp palindrome in *Tatl* is double-underlined.

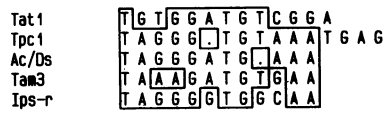

FIG. 3. Comparison of the inverted repeats of the transposons *Tat1* of *A. thaliana*, *Tpc1* of *Pe. crispum* (9), *Ac/Ds* of *Z. mays* (7), *Tam3* of *An. majus* (8), and *Ips-r* of *Pi. sativum* (10).

with the *Tat1a* and *Tat1c* sequences, respectively. *Tat1c* is 431 bp long and shows 95.5% identity with *Tat1a*. The terminally inverted repeats are completely conserved in the three isolates. The *Tat1a* and *Tat1b* isolates are flanked by different direct repeats, respectively ATCGT (*Tat1a*) and TGAAC (*Tat1b*). By contrast the *Tat1c* isolate is not flanked by a direct repeat.

Genomic Southern Analysis. To determine whether the *Tat1* element is present in multiple copies in the *Arabidopsis* genome, the element was hybridized with DNA prepared from small populations of different ecotypes and collection numbers of *A. thaliana* (Wassilewskija, Landsberg *erecta*, Columbia, C24, and K85). The DNA was digested with different enzymes and hybridized with the 0.5-kb *Bam*HI–*Xho* I fragment containing the *Tat1b* isolate as a probe (Fig. 4). For each digest, multiple bands (4 to >10) with different intensities appeared, indicating that the *Arabidopsis* genome contains several copies of the *Tat1* element. The hybridization patterns are very different between the different *Arabidopsis* ecotypes with all of the enzymes tested. By contrast, only ≈20% of randomly chosen cosmid clones of *Arabidopsis* containing DNA with a low-copy number show restriction fragment-length polymorphism between Columbia and Landsberg *erecta* ecotypes when using one restriction enzyme (17). Therefore, the genomic positions of at least some of the *Tat1* elements must have changed since the divergence of the different tested *Arabidopsis* ecotypes.

The poorly hybridizing bands may represent DNA fragments with low or dispersed homology. If we consider the sequences in the bands giving strong hybridization signals as being highly homologous to *Tat1*, we estimate that a haploid *A. thaliana* ecotype Columbia genome contains at least four copies of the *Tat1* element. However, the number of hybridizing bands seems to vary in the different ecotypes.

DNA from other genera than *Arabidopsis* (*Brassica*, *Nicotiana*, *Oryza*) does not hybridize with the *Tat1* element (data not shown).

Identification of Extrachromosomal *Tat1* DNA. To examine the distribution of the *Tat1* element in the genome of single plants, DNA was prepared from 24 individual plants grown from the seeds of one self-fertilized *Arabidopsis* ecotype Columbia plant. Subsequently, *Bgl* II digests of these DNAs were hybridized with the 0.5-kb *Bam*HI–*Xho* I fragment containing the *Tat1b* isolate. The hybridization pattern of eight of these plants is shown in Fig. 5*A*. Four strongly hybridizing bands of 10, 5.0, 4.0, and 2.0 kb appeared in all plants. A band of ≈0.4 kb, hybridizing with variable intensity, was visible in only 6 of the 24 plants. The nature of this band, with a size in the range of that of the *Tat1* element, was examined more closely on a 1.5% agarose gel. When undigested DNA of plant 3 (Fig. 5*B*) was hybridized with the *Tat1* element as a probe, a 0.43-kb band was still present, indicating that this DNA is extrachromosomally present. Judging from the relative hybridization intensities of this band, the extrachromosomal *Tat1* DNA is present in variable concentrations. In several plants this extrachromosomal *Tat1* DNA is present at a higher concentration than the chromosomal *Tat1*-hybridizing bands. Despite this, none of the chromosomal *Tat1* bands has disappeared, suggesting that there is an amplification step involved in the generation of this extrachromosomal *Tat1* DNA.

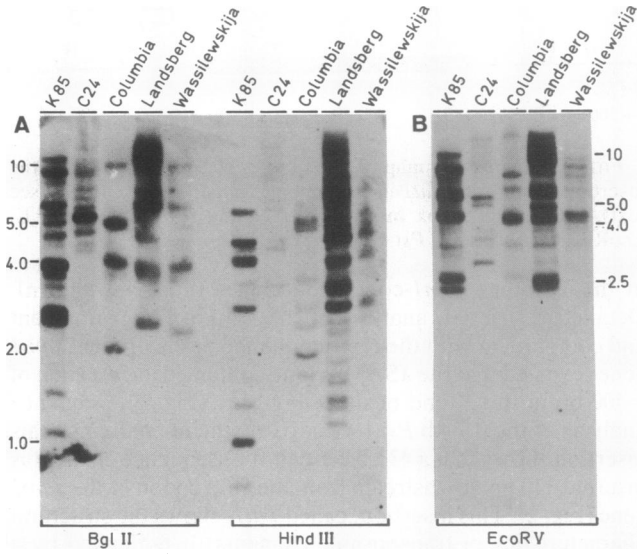

FIG. 4. Genomic Southern hybridizations with the *Tat1* element as a probe. *Bgl* II, *Hin*dIII, and *Eco*RV digests of total DNA prepared from different ecotypes of *A. thaliana* (K85, C24, Columbia, Landsberg *erecta*, and Wassilewskija) were hybridized with the 0.5-kb *Bam*HI–*Xho* I fragment of pTAT1B-2 (*Tat1b* isolate) as a probe.

To determine whether the extrachromosomal *Tat1* DNA is present in a linear or circular form, plant 3 DNA was digested with *Apa* I. This enzyme cuts in the middle of the 20-bp palindrome of *Tat1*, which is located 65 nucleotides from the left border of the *Tat1* element (Fig. 2). After hybridization with the *Tat1* element as a probe, there is a 0.36-kb band present instead of the 0.43-kb band. This observation shows that the extrachromosomal *Tat1* DNA is present as a linear molecule.

To examine whether the extrachromosomal *Tat1* DNA is transmitted through meiosis, progenital plants were grown after self-fertilization of the plants. From this progeny, a population of ≈15 plants was used for DNA preparation and subsequent hybridization analysis. The hybridization patterns are compared with those of the parental plants in Fig. 5*A*. None of the progenital plants contains the 0.43-kb extrachromosomal *Tat1* DNA. Moreover, no new chromosomal locations of the *Tat1* element can be detected.

To find new populations of plants containing extrachromosomal *Tat1* DNA, DNA was prepared from the progeny of 35 independently *in vitro* regenerated *A. thaliana* collection number C24 plants. Tissue culture-derived plants were used because there are indications that tissue culture may activate transposable elements (for a review, see ref. 18). For each DNA preparation, 15–20 individuals were harvested. Subsequently, undigested, *Bgl* II-digested and *Apa* I-digested DNA of these populations was hybridized with the *Tat1* element as a probe (Fig. 5*C*). Only one population (R14) contained *Tat1* homologous extrachromosomal DNA. This extrachromosomal DNA is ≈850 nucleotides long. Digestion of the DNA of this population with *Apa* I reduced the length of this fragment to 700 nucleotides. This observation indicates that this extrachromosomal DNA consists of a dimer of the *Tat1* element oriented in an inverted repeat. However, this dimer is not present in the genome of these plants, since the other examined populations of the same variety do not show a 700-nucleotide-long hybridizing band after *Apa* I digestion. Therefore, the dimer must have been generated during or after the process of excision.

DISCUSSION

In this paper, the sequence is presented of three independent isolates of a 430-bp insertion element of *A. thaliana*, called

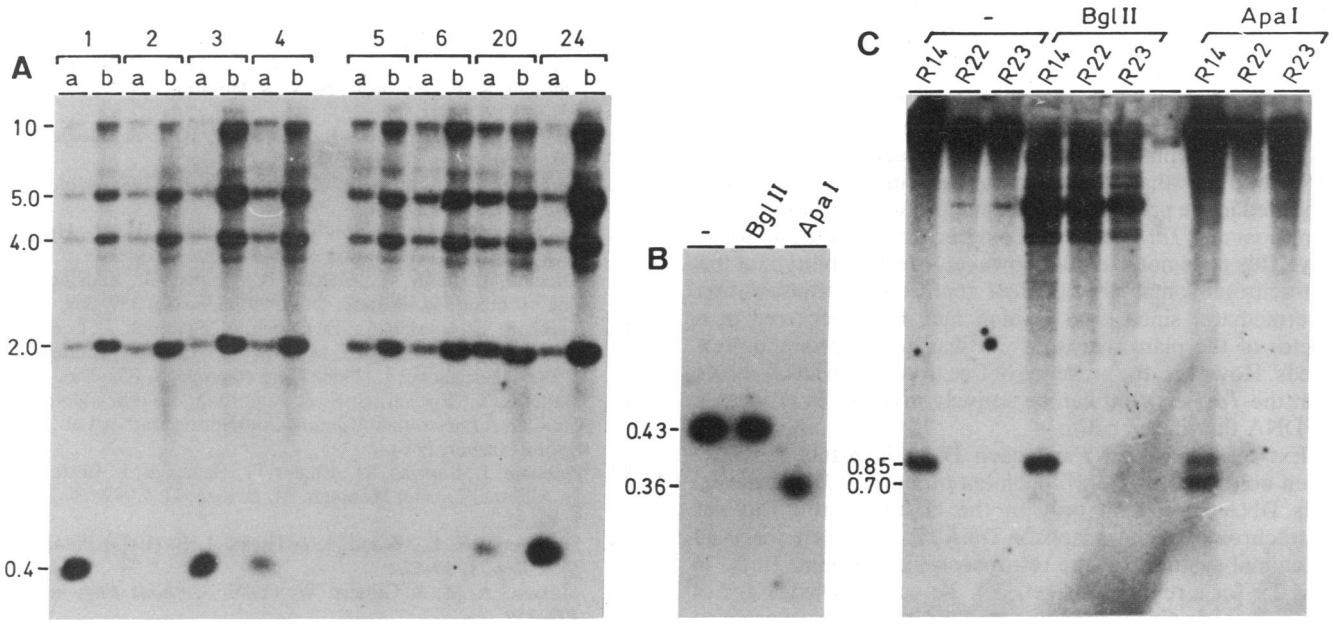

FIG. 5. Detection of extrachromosomal *Tat1* DNA by genomic Southern hybridizations. (*A*) Genomic Southern analysis of eight individual plants and their progenies. Total DNA was prepared from single *A. thaliana* ecotype Columbia plants grown from the seeds of one self-fertilized plant. After *Bgl* II digestion and gel electrophoresis, the DNA was hybridized with the 0.5-kb *Bam*HI–*Xho* I fragment of pTAT1B-2 as a probe (lanes a). In the same hybridization experiment, *Bgl* II-digested DNA was included which was prepared from small populations (±15 plants) of the different progenies obtained after self-fertilization of the individual plants. After *Bgl* II digestion, the DNAs were loaded next to the respective parental DNA (lanes b). (*B*) Southern analysis of the 0.43-kb extrachromosomal *Tat1* homologous DNA. DNA obtained from plant 3 (see *A*, lane 3a) was run on a 1.5% agarose gel and hybridized with 0.5-kb *Bam*HI–*Xho* I fragment of pTAT1B-2. Only the lower part of the filter containing the extrachromosomal DNA is shown. Lane "−" contains undigested DNA. (*C*) Detection and analysis of 0.85-kb extrachromosomal *Tat1*-homologous DNA. Total DNA was prepared from small populations (15–20 individuals) of *in vitro* grown *A. thaliana* (collection number C24) plants. This DNA was run on a 0.8% agarose gel in the undigested (lanes −), *Bgl* II-digested, or *Apa* I-digested form and was hybridized with the 0.5-kb *Bam*HI–*Xho* I fragment of pTAT1B-2.

Tat1. *Tat1* has two structural characteristics of transposable elements: the presence of a 5-bp duplication of the target site DNA flanking the element and a 13-bp inverted repeat at the ends of the element. This inverted repeat sequence shows homology with the inverted repeats of *Ac/Ds* elements in maize, *Tam3* in *An. majus*, and *Tpc1* of *Pe. crispum* (7–9). *Tat1* does not show striking similarities with the *Tal* retroposon-like element that has been isolated from *Arabidopsis* (19). Genomic Southern hybridization with *Tat1* indicates that there are at least four copies of *Tat1* present per haploid genome of *A. thaliana* ecotype Columbia.

The *Tat1* elements do not contain any significant open reading frame and, therefore, are unlikely to transpose autonomously. Possibly, *Tat1* is, like many *Ds* elements in maize, a defective deletion derivative of a larger element (7, 20). In this context, however, it is atypical that the three different isolates of *Tat1* presented in this paper have almost exactly the same length and that they are highly homologous. Maybe, *Tat1* is an independent element that moves within the genome by utilizing existing nuclear enzymes. On the other hand, *Tat1* may represent a long terminal repeat (LTR) of a retroposon-type element. Solitary LTRs can originate from a retroposon by direct recombination between the terminal LTRs. Solo *delta* sequences in yeast, for example, are believed to originate by recombination between the LTRs of the *Ty* element (21, 22). It should be mentioned here that the two external base pairs of *Tat1* are 5′ TG---CA 3′, which is characteristic for retroposon-type elements. However, the flanking regions of the different *Tat1* isolates do not show significant homology with any known retroposon-type elements.

Despite the apparent inability of *Tat1* to transpose autonomously, there are some lines of evidence indicating that *Tat1* is an actively transposing element. Genomic hybridizations show that the *Tat1* copies have changed their positions in the genome at least since the divergence of the different ecotypes of *Arabidopsis* tested (Wassilewskija, Landsberg *erecta*, Columbia, C24, and K85). Moreover, within one ecotype of *A. thaliana* there seems to exist heterogeneity in the positions of the *Tat1* element. Within 11 *sam1* gene isolates from a genomic library prepared from a population, only 1 *sam1* isolate contained a *Tat1* insertion in its 3′ end. This observation was confirmed in genomic Southern analysis with *Arabidopsis* DNA from the same ecotype as the library was derived from. A probe containing the *Tat1* element plus 288 bp of flanking sequences from the 3′ end of *sam1* gave a stronger hybridization signal with *sam1* DNA fragments lacking the *Tat1* insertion than those containing *Tat1* (data not shown). The sequences flanking the *Tat1* insertion in the 3′ end of *sam1* are 100% conserved with the 3′ end sequences of the *sam1* isolates without *Tat1* insertion, indicating that the *Tat1* insertion has occurred recently.

A particular feature of the *Tat1* element is the occurrence of linear extrachromosomal *Tat1*-homologous DNA, which has been observed in 2 of 36 examined populations of *A. thaliana*. The first examined population consisted of 24 plants (ecotype Columbia) derived from the seeds of one self-fertilized parental plant. Six of these plants contained extrachromosomal *Tat1*-homologous DNA. This high-occurrence frequency of this state within one progeny suggests that the generation of the extrachromosomal *Tat1* DNA has been initiated in the parental plant. The concentration of the extrachromosomal *Tat1* DNA was variable and in some plants was higher than one copy per genome. Therefore, there must be an amplification step involved in the generation of this free *Tat1* DNA. Another population of plants (collection number C24) contained 850-nucleotide-long *Tat1*-homologous extrachromosomal DNA. Restriction and subsequent hybridization analysis indicated that this DNA consisted of a dimer of the *Tat1* element in inverted orientation. Since this dimer as such is not present in the genome of the

corresponding *Arabidopsis* ecotype, it must have been formed during or after the generation of the extrachromosomal *Tat1* DNA.

It is unclear how this extrachromosomal *Tat1* DNA is generated and what its possible function is. Analysis of the progeny of the plants containing the extrachromosomal *Tat1* DNA showed that this extrachromosomal DNA was not inherited in the progeny of these plants. Additionally, no new chromosomal *Tat1* positions could be observed in the progeny. This does not exclude, however, the possibility that the extrachromosomal state of *Tat1* represents a transposition intermediate, since transposition may have occurred in a sector of the plant that does not lead to the production of seeds. However, the presence of free transposon DNA shows that the *Tat1* element can be actively involved in processes of DNA flux in the cell.

Extrachromosomal transposon DNA in plants has only been observed for the *Mu* element of maize (23). However, this DNA is present only in the circular form. Circular extrachromosomal transposon DNA has also been observed in animal systems for the retroposon-like elements (refs. 24 and 25; for a review, see ref. 26). To our knowledge *Tc1* of the nematode *Caenorhabditis elegans* is the only transposon for which the presence of a linear extrachromosomal form has been reported in addition to a circular form (27).

We thank J. Seurinck and J. Gielen for sequence determination; Dr. A. Caplan for careful reading of the manuscript; M. De Cock for typing it; K. Spruyt, V. Vermaercke, and S. Van Gijsegem for figures and photographs; and the Belgian Army for delaying the work during 1 year. This work was supported by grants from the Services of the Prime Minister (U.I.A.P. 120C0187) and the Algemene Spaar-en Lijfrentekas–Kankerfonds. J.P. and W.V.C. are indebted to the Instituut ter Aanmoediging van het Wetenschappelijk Onderzoek in Nijverheid en Landbouw for a predoctoral fellowship. D.I. was Senior Research Assistant of the National Fund for Scientific Research (Belgium).

1. Döring, H.-P. & Starlinger, P. (1986) *Annu. Rev. Genet.* **20,** 175–200.
2. Fedoroff, N. (1989) in *Mobile DNA*, eds. Howe, M. M. & Berg, D. E. (Am. Soc. Microbiol., Washington, DC), pp. 377–411.
3. Hehl, R., Sommer, H. & Saedler, H. (1987) *Mol. Gen. Genet.* **207,** 47–53.
4. Vodkin, L. O., Rhodes, P. R. & Goldberg, R. B. (1983) *Cell* **34,** 1023–1031.
5. Schwarz-Sommer, Zs., Gierl, A., Klösgen, R. B., Wienand, U., Peterson, P. A. & Saedler, H. (1984) *EMBO J.* **3,** 1021–1028.
6. Shirsat, A. H. (1988) *Mol. Gen. Genet.* **212,** 129–133.
7. Pohlman, R. F., Fedoroff, N. V. & Messing, J. (1984) *Cell* **37,** 635–643.
8. Sommer, H., Carpenter, R., Harrison, B. J. & Saedler, H. (1985) *Mol. Gen. Genet.* **199,** 225–231.
9. Herrmann, A., Schulz, W. & Hahlbrock, K. (1988) *Mol. Gen. Genet.* **212,** 93–98.
10. Bhattacharyya, M. K., Smith, A. M., Ellis, T. H. N., Hedley, C. & Martin, C. (1990) *Cell* **60,** 115–122.
11. Peleman, J., Saito, K., Cottyn, B., Engler, G., Seurinck, J., Van Montagu, M. & Inzé, D. (1989) *Gene* **84,** 359–369.
12. Krebbers, E., Herdies, L., De Clercq, A., Seurinck, J., Leemans, J., Van Damme, J., Segura, M., Gheysen, G., Van Montagu, M. & Vandekerckhove, J. (1988) *Plant Physiol.* **87,** 859–866.
13. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
14. Peleman, J., Boerjan, W., Engler, G., Seurinck, J., Botterman, J., Alliotte, T., Van Montagu, M. & Inzé, D. (1989) *Plant Cell* **1,** 81–93.
15. Dellaporta, S. L., Wood, J. & Hicks, J. B. (1983) *Plant Mol. Biol. Rep.* **1,** 19–21.
16. Maxam, A. M. & Gilbert, W. (1980) *Methods Enzymol.* **65,** 499–559.
17. Nam, H.-G., Giraudat, J., den Boer, B., Moonan, F., Loos, W. D. B., Hauge, B. M. & Goodman, H. M. (1989) *Plant Cell* **1,** 699–705.
18. Lee, M. & Phillips, R. L. (1988) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **39,** 413–437.
19. Voytas, D. F. & Ausubel, F. M. (1988) *Nature (London)* **336,** 242–244.
20. Döring, H.-P., Tillman, E. & Starlinger, P. (1984) *Nature (London)* **307,** 127–130.
21. Cameron, J. R., Loh, E. Y. & Davis, R. W. (1979) *Cell* **16,** 739–751.
22. Kingsman, A. J., Mellor, J., Adams, S., Rathjen, P. D., Malim, M. H., Fulton, S. M., Wilson, W. & Kingsman, S. M. (1987) *J. Cell Sci. Supp.* **7,** 155–167.
23. Sundaresan, V. & Freeling, M. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 4924–4928.
24. Flavell, A. J. & Ish-Horowicz, D. (1981) *Nature (London)* **292,** 591–595.
25. Mossie, K. G., Young, M. W. & Varmus, H. E. (1985) *J. Mol. Biol.* **182,** 31–43.
26. Weiner, A. M., Deininger, P. L. & Efstratiadis, A. (1986) *Annu. Rev. Biochem.* **55,** 631–661.
27. Ruan, K.-S. & Emmons, S. W. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 4018–4022.