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Insertion sequence elements of *Pseudomonas savastanoi*: Nucleotide sequence and homology with *Agrobacterium tumefaciens* transfer DNA

(plant tumorigenicity/transposition/tryptophan monooxygenase/indoleacetamide hydrolase)

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ABSTRACT **Two types of transposable elements, IS*51* and IS*52* (IS, insertion sequence), were found in *Pseudomonas syringae* subsp. *savastanoi* (*P. savastanoi*) that spontaneously insert into and inactivate *iaaM*; the insertion results in the loss of indoleacetic acid production and attenuation of virulence. The nucleotide sequences of both IS elements have sizes and structural features common to other prokaryotic IS elements; IS*51* is 1311 base pairs (bp) long and has terminal inverted repeats of 26 bp; IS*52* is 1209 bp long and has terminal inverted repeats of 10 bp with a 1 bp mismatch. In the insertion involving IS*51*, the trinucleotide sequence CAG is duplicated within *iaaM* sequences at the recombination junction; in those involving IS*52* the tetranucleotide sequences TTAG or CTAG are duplicated within *iaaM* sequences at the recombination junction. A copy of IS*51* occurs 2.5 kilobases downstream from *IaaH*. In contrast to the high copy number of IS*51* in the genome of the bacterium, only a few copies of IS*52* are present. No nucleotide sequence homology was found between IS*51* and IS*52*. However, a striking nucleotide sequence homology was found between a 531-bp region of IS*51* and a portion of the central region of transfer DNA (T-DNA) in the octopine plasmid pTi15955 from *Agrobacterium tumefaciens*. These observations, together with our earlier finding on the homology between *iaaM* and *iaaH* and between gene 1 and gene 2 of transfer DNA, further suggest that genes for indoleacetic acid production in the two systems have a common origin.**

The bacterium *Pseudomonas syringae* subsp. *savastanoi* (*P. savastanoi*) incites the production of galls (knots) at the site of infection on olive and oleander plants. Tumor formation by these plants is a response to high concentrations of indoleacetic acid (IAA) introduced into infected tissue by the bacterium (1). The bacterium produces IAA from tryptophan with indoleacetamide (IAM) as the intermediate (2). The enzymes involved are tryptophan 2-monooxygenase, which catalyzes the conversion of L-tryptophan to IAM, and IAM hydrolase, which catalyzes the conversion of IAM into ammonia and IAA (3). The genetic determinants for the two enzymes, *iaaM* and *iaaH*, respectively, are part of an operon that is borne on a plasmid, pIAA, in oleander strains of the pathogen; in olive strains the determinants are on the chromosome.

Earlier, we showed similarities between the *P. savastanoi* and crown gall systems for IAA synthesis (4). Nucleotide sequences of *iaaM* and *iaaH* show significant homology with sequences of genes 1 and 2 of the transfer DNA (T-DNA) in *A. tumefaciens* (4). Genes 1 and 2 of T-DNA encode enzymes functionally similar to the tryptophan monooxygenase and IAM hydrolase from *P. savastanoi* (5–8).

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We report here the characterization of two insertion sequence (IS) elements, IS*51* and IS*52*, found in avirulent mutants of *P. savastanoi* deficient in IAA production (9). The IS elements are of interest because they were responsible for loss of IAA production and virulence by being integrated into *iaaM* (3, 9). The nucleotide sequences of these IS elements were determined. Strong homology was found between IS*51* and a 528-base-pair (bp) region within the central region of the T-DNA (T_c) of *A. tumefaciens*. The results suggest that the homologous border sequence may be a remnant of an earlier transpositional event by which the genes for IAA synthesis were transferred between the two species of tumorigenic bacteria.

MATERIALS AND METHODS

Bacterial Strains, Plasmids, and Media. Strains of bacteria, plasmids, culture conditions, and media have been described (4, 9). Spontaneous Iaa$^-$ mutants were isolated by selection for α-methyltryptophan resistance (3).

DNA Isolation. Procedures for plasmid and total DNA isolation, restriction endonuclease digestions, and electrophoresis in agarose gels were described (1).

Transfer of DNA to Filters and Hybridization. DNA fragments were transferred from agarose gels to nitrocellulose filters (Schleicher & Schuell) by the Southern blot procedure (10). Hybridization was performed at 68°C as described by Maniatis *et al.* (11).

Labeling of DNA. DNA preparations were labeled by nick-translation (11), with DNase I (Boehringer Mannheim), [α-^{32}P]dATP (Amersham), and DNA polymerase I (New England Biolabs). Labeled DNA was separated from the unincorporated nucleotides by a nitrocellulose column (Bethesda Research Laboratories; NACS PREPAC) (12).

Extraction of DNA from Agarose Gels. Endonuclease-digested DNA preparations were resolved by electrophoresis on 1.0–1.2% low-temperature-melting agarose gels. Gel slices containing the DNA fragments were put in appropriate tubes with the elution buffer [0.5 M NaCl/10 mM Tris Cl, pH 7.5/1 mM EDTA] and incubated at 70°C until the agarose melted completely. DNA was removed by passage through a column of NACS nitrocellulose (12).

Ligation and Transformation of DNA. DNA was ligated with T4 DNA ligase (New England Biolabs) by the procedures of Maniatis *et al.* (11). Procedures for storage and transformation of competent cells were described (1).

Cloning of IS Elements. Fragment Ω2M (9), bearing IS*52* from strains PB205-1L and TK2015-3 (Fig. 1), was inserted into the *Eco*RI site in the vector pBR328 and resulting

Abbreviations: T-DNA, transfer DNA—DNA transferred from the Ti plasmid to a plant cell; T_c, center region of T-DNA; kb, kilobase(s); bp, base pair(s); IS, insertion sequence; IAA, indoleacetic acid; IAM, indoleacetamide; ORF, open reading frame.

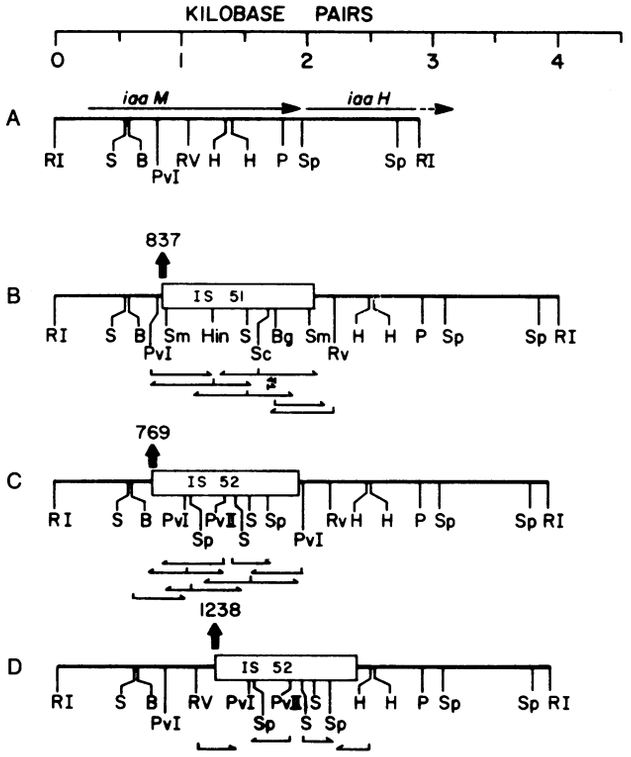

FIG. 1. Restriction maps of fragment M or fragment ΩM (9) isolated from different strains of *P. savastanoi* and sequencing strategy used for nucleotide sequencing of IS*51* and IS*52*. Positions of *iaaM* and *iaaH* in fragment M are shown. Horizontal arrows below the boxes show the extent and direction of sequence analysis. (*A*) Fragment M in pLUC2 (wild type, TK2009). (*B*) Fragment Ω1M in pLUC12 (TK2009-5). (*C*) Fragment Ω2M in pTET1 (PB205-1L). (*D*) Fragment Ω2M in pTET11 (TK2015-3). The boxed area represents the inserted DNA. B, *Bam*HI; Bg, *Bgl* II; H, *Hin*dIII; P, *Pst* I; PvI, *Pvu* I; PvII, *Pvu* II; RI, *Eco*RI; RV, *Eco*RV; S, *Sal* I; Sc, *Sac* I; Sm, *Sma* I; Sp, *Sph* I; Hin, *Hin*cII. The numbers above the solid arrows indicate the position of nucleotides from the *Eco*RI site at the 5′ end of fragment M (4).

recombinant plasmids were transformed into *Escherichia coli* HB101. Both orientation isomers of pBR328::fragment Ω2M hybrids from PB205-1L and TK2015-3 were found, and the ones in which *iaaM* transcription was oriented with the transcription of chloramphenicol resistance gene (i.e., clockwise) were designated pTET1 and pTET11, respectively.

Nucleic Acid Sequencing. The recombinant plasmids pLUC12 (9), pTET1, and pTET11 bearing *iaaM* insertionally inactivated by IS*51* and IS*52* provided the DNA to be sequenced (Fig. 1). The plasmids were digested with selected endonucleases, and the resulting restriction fragments were cloned into the M13 vectors, mp8, mp11, mp18, or mp19 (13). After transformation into *E. coli* 7118, white plaques were screened by the method of Messing and Vieira (14). Phage clones of both strands of the fragments bearing the IS element and border regions were isolated for nucleotide sequencing. A single-stranded phage template was prepared by the procedure of Messing (13) and used for the dideoxy sequencing reactions described by Sanger *et al.* (15); dATP[α-^{35}S] (Amersham) (16) was used, and electrophoresis for nucleotide sequencing was carried out in 8% polyacrylamide (Bethesda Research Laboratories model 50; 34 × 40 cm, 0.2 mm thick) or 6% polyacrylamide (Bethesda Research Laboratories model S1; 30 × 84 cm; 0.2 mm thick).

RESULTS

Restriction Maps of the IS Elements and Their Flanking Regions. To characterize the IS element(s) found in strains PB205-1L and TK2015-3, fragment Ω2M from pTET1 and from pTET11 was mapped by single and double digestion with different restriction endonucleases. Since both putative IS elements from PB205-1L and TK2015-3 have the same restriction sites *Pvu* I, *Pvu* II, *Sph* I, and *Sal* I and were similar in size (Fig. 1), the two appear to be identical, and clearly distinct from IS*51*.

Occurrence of IS*52* on Plasmids and Chromosome. To determine the distribution of the IS*52* in the genome of different strains, ^{32}P-labeled plasmid pTET1 was hybridized to DNA preparations bound to nitrocellulose filters. Purified plasmid DNA that had been digested with *Eco*RI was fractionated through an agarose gel and transferred to nitrocellulose filters (Fig. 2*A*). Hybridization with pTET1 was observed in plasmid digests of strains carrying pIAA1 (TK2009, TK2015-3) at the positions of fragment M (M) or fragment Ω2M (Ω2M), which involve *iaaM* sequence (Fig. 2*B*). On the other hand, strain PB205-1L carrying pIAA2 (9) had another plasmid-borne sequence homologous to IS*52*. Strains TK2009 and PB205-1L each showed at least five chromosomal fragments that hybridized with IS*52* (data not shown).

A Copy of IS*51* Occurs in Wild-Type pIAA1. Comai and Kosuge (9) had shown that IS*51* has several characteristic restriction sites. The same pattern of restriction sites occurred in pIAA1 approximately 2.5 kilobases (kb) downstream from the end of *iaaH* (C. Palm, personal communication) in wild-type strain TK2009. To determine if the cleavage pattern is related to the occurrence of IS*51*, *Pst* I digests of pLUC14 (9.7-kb *Hin*dIII fragment of pIAA1 carrying *iaaH* and 7.7-kb downstream region cloned in the vector pBR328) (Fig. 3*A*) was hybridized with a single-stranded DNA, M13mp8 clone carrying a 2.0-kb insert containing IS*51* (Fig. 3*B*); after hybridization the mixture was digested with S1 nuclease (11). The identical protocol of hybridization and S1-nuclease digestion was employed with a second sample containing *Eco*RI digests of pLUC14 and M13mp8 clone. Hybridized fragments protected from S1 digestion were detected by agarose gel electrophoresis of S1-nuclease digestion mixtures. In both samples, a 1.3-kb fragment corresponding in size to IS*51* was generated (Fig. 3*C*). Moreover, the 1.3-kb fragment had *Bgl* II and *Sal* I sites in positions identical to those found in IS*51* (data not shown). To further confirm if the fragment generated by S1 nuclease is IS*51*, ^{32}P-labeled plasmid pLUC12 was hybridized to S1-nuclease digests (Fig. 3*D*). Only pLUC12 hybridized with the 1.3-kb fragment (Fig. 3*D*, lane F); no hybridization

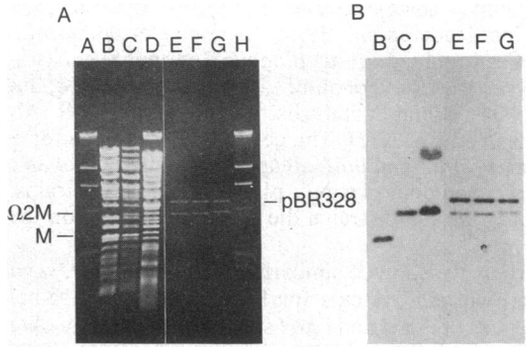

FIG. 2. (*A*) Agarose gel electrophoresis of endonuclease *Eco*RI digests of the total plasmids isolated from TK2009 (lane B), TK2015-3 (lane C), PB205-1L (lane D), and recombinant plasmid pTET1 (lane E), pTET11 (lane F), and pLUC12 (lane G). Location of fragment M (M), fragment Ω2M (Ω2M) (9), and vector pBR328 are shown. Lanes A and H, *Hin*dIII digests of λ DNA. (*B*) Southern hybridization of lanes B–G with ^{32}P-labeled pTET1 that contains IS*52*. The lanes correspond to those in Fig. 2*A*.

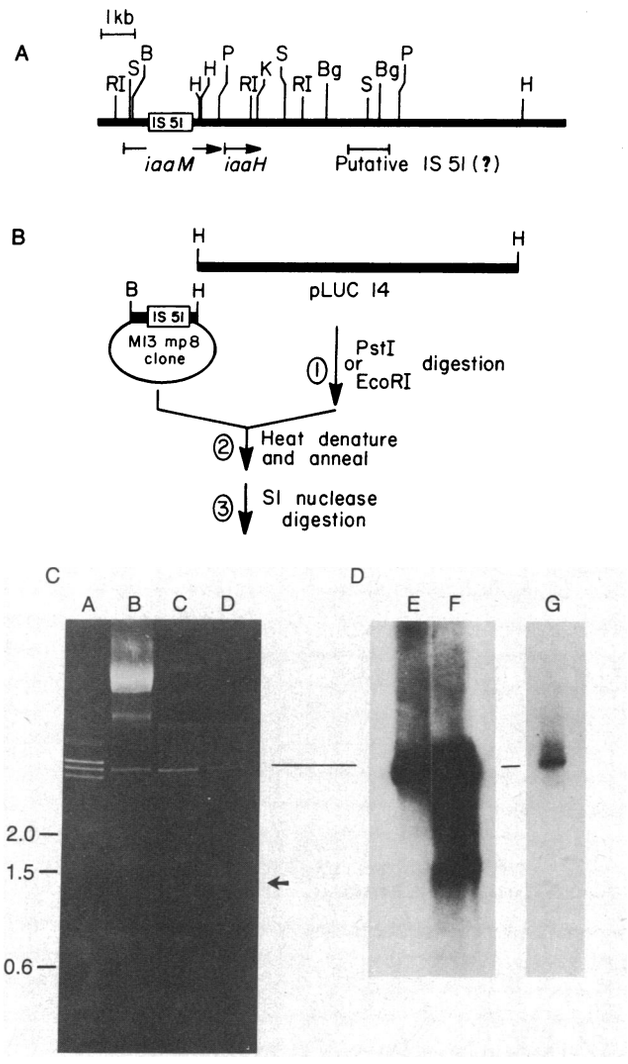

FIG. 3. Schematic presentation of S1-nuclease digestion to generate repetitive sequences of IS*51*. (*A*) Partial restriction map of pIAA plasmid carrying *iaa* genes and the 9.7-kb *Hin*dIII fragment contained in pLUC14. Arrows indicate the position of *iaaM* and *iaaH*. The location of a putative IS*51* is also shown. (*B*) A map of a single stranded DNA carrying a 2.0-kb fragment obtained by *Bam*HI–*Hin*dIII double digestion of fragment Ω1M (9) cloned in M13mp8 DNA. The solid line flanking to IS*51* is a part of *iaaM* fragment. B, *Bam*HI; Bg, *Bgl* II; H, *Hin*dIII; K, *Kpn* I; P, *Pst* I; RI, *Eco*RI; S, *Sal* I. (*C*) Agarose gel electrophoresis of samples digested with S1 nuclease. Lane A, *Pst* I digests of pLUC14. Lanes B–D, S1-nuclease digests of the hybridized mixtures of M13 clone and *Pst* I digests of pLUC14 (see text); no addition of S1 nuclease (lane B), 40 units (lane C), and 60 units (lane D) of S1 nuclease. The arrow marks the position of a 1.3-kb IS*51*-like fragment. The numbers on the left give the positions of the molecular size standards (in kb). (*D*) Southern hybridization of S1-nuclease digests to ^{32}P-labeled probes. Lanes E and F, S1-nuclease digests shown in lanes B and C in *C* were blotted onto the nitrocellulose filter and hybridized with ^{32}P-labeled pLUC12, which bears IS*51*. Lane G, S1-nuclease digests shown in lane C in *C* were hybridized with ^{32}P-labeled pTET1, which bears IS*52*.

occurred with ^{32}P-labeled pTET1 that contains IS*52* (Fig. 3*D*, lane G). These results strongly suggest that a sequence homologous to IS*51* is present approximately 2.5 kb downstream from *iaaH* gene and further indicate that IS*52* in pTET1 (PB205-1L) is distinct from IS*51*.

Nucleotide Sequences of IS*51* and IS*52*. The nucleotide sequences of IS*51*, IS*52*, and their flanking regions were determined. IS*51* is 1311 bp long and has terminal inverted repeats of 26 bp; additional 6-bp inverted repeats were found after 6- or 7-bp mismatches (Fig. 4*A*). A 3-bp sequence, CAG, at the target sequence in *iaaM* is duplicated at both recombination junctions. Open reading frames (ORFs) of 357 bp and of 258 bp, designated ORF1 and ORF2, respectively, were found in one strand and ORFs of 321 bp (ORF3) and of 900 bp (ORF4) were found in the opposite strand. Several ORFs of 200 bp are present in both strands. As noted in position 950 (Fig. 4*A*), dinucleotides TG in the stop codon (TGA) in ORF3 overlapped with the start codon (ATG) of the longest ORF ORF4, which could be a transposase structural gene. Moreover, based upon sequence similarities with *Pseudomonas aeruginosa* (17), a possible ribosomal binding sequence, AGAG, was found 8 bp upstream from the start codon of ORF3; however, no such sequences are present within 30 bp upstream in ORF1, ORF2, or ORF4.

IS*52* from PB205-1L is 1209 bp long and has terminal inverted repeats of 10 bp with 1-bp mismatches (Fig. 4*B*). A 4-bp sequence, TTAG, of the target sequence is duplicated at both recombination junctions. ORFs of 768 bp (ORF1), 264 bp (ORF2), and two 237 bp (ORF3, ORF4) were found in one strand, and 738 bp (ORF5) was in the opposite strand. Several ORFs of 200 bp are present on each strand. No consensus sequence characteristic of ribosome binding sites was found within 30 bp upstream of the start codon of any ORFs. Approximately 80% of the nucleotide sequence of the IS element in TK2015-3 was also determined (Fig. 1); it showed perfect homology with the sequences of IS*52* in PB205-1L. No significant sequence homology was found between IS*51* and IS*52*.

Nucleotide Sequences at the Target Sites. Nucleotide sequences of the target duplications made by IS elements at recombinant junctions in pLUC12 (IS*51*), pTET1 (IS*52*), and pTET11 (IS*52*) were CAG, TTAG, and CTAG, respectively (Fig. 4), according to the nucleotide sequence of the *iaaM* (4). It is likely that during integration IS*51* makes a 3-base staggered nick and IS*52* makes a 4-base staggered nick at the target sites. However, interestingly enough, both IS elements appear to have the "hot spot" sequences of YAG at their target recombination sites. No obvious consensus sequences were found in the flanking regions of the IS elements target sites apart from the target duplications.

Homology Between IS*51* and the T_c Region of T-DNA. When we compared the nucleotide sequences of IS*51* and IS*52* with published sequences compiled by the Microgenie Programs (Beckman), we found striking homology between IS*51* and T_c region of the *A. tumefaciens* T-DNA (18) (Fig. 5). A portion of IS*51*, within positions 9–539 in Fig. 4*A*, including the integration site sequence AG, has a homology of 67.7% with sequences from position 15,614 through 15,087 in the T_c region of T-DNA from the octopine Ti plasmid pTi15955 (18). These sequences in IS*51* correspond to half of the largest ORF at the carboxyl terminus; however, T-DNA contains two ORFs, 459 bp and 462 bp, in this homologous region.

DISCUSSION

Two types of translocatable DNA elements, IS*51* and IS*52*, can integrate into and inactivate a bacterial gene, *iaaM*, which encodes an enzyme concerned with virulence in the plant pathogen, *P. savastanoi*. In confirmation of previous hybridization experiments, at least 5 sequences homologous to IS*51* were found in digests of plasmids, and up to 20 in digests of the chromosome of different strains of *P. savastanoi* (9). However, the wild-type TK2009, which carries pIAA1, had only chromosome-borne copies of IS*52*, which suggests that the transposition of IS*52* into *iaaM* in Iaa$^-$ mutant, TK2015-3, occurred from the chromosome. Although all oleander strains of *P. savastanoi* so far examined contain both IS*51* and IS*52*, the number of copies of IS*51* is much higher than IS*52*.

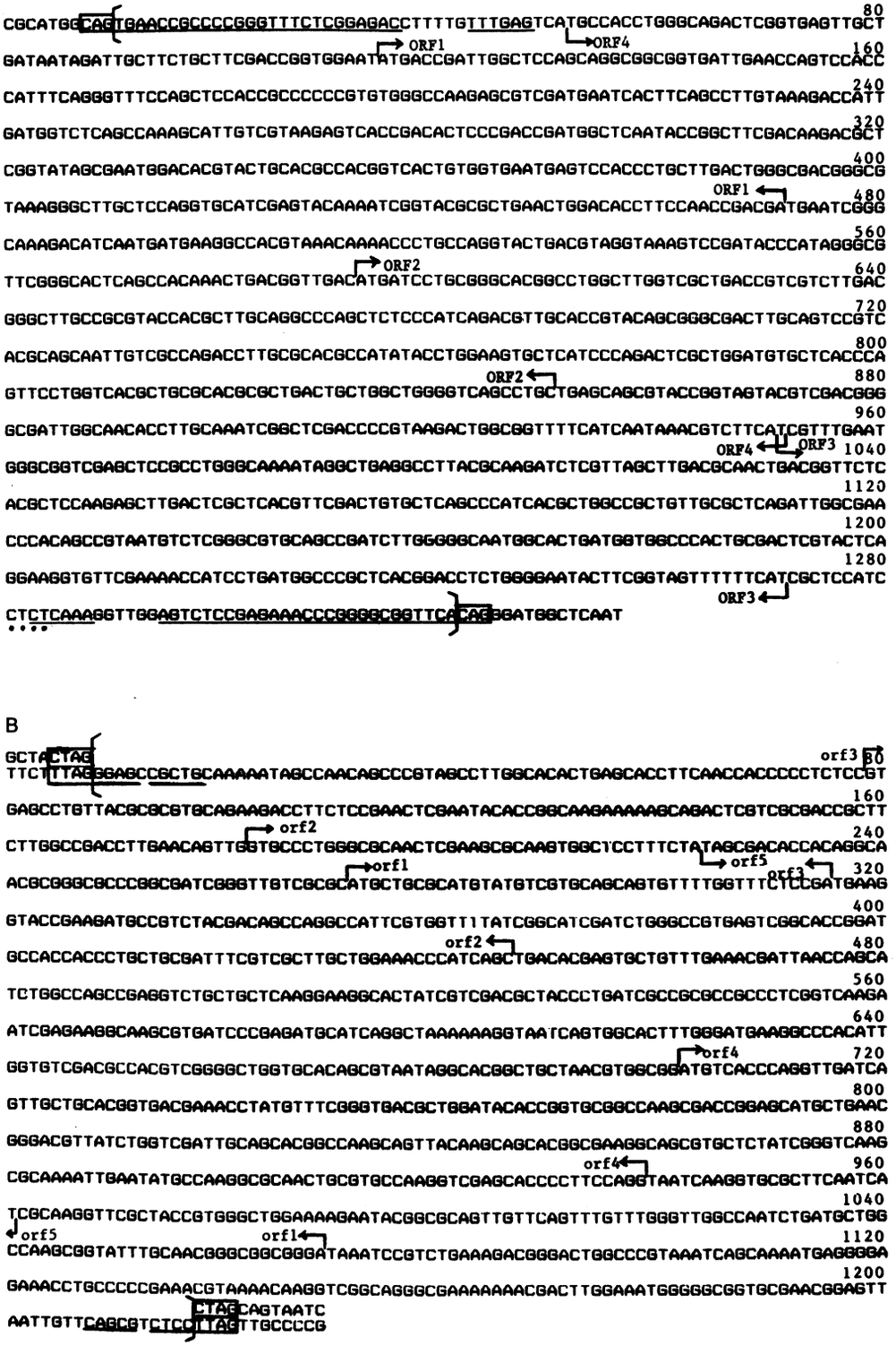

FIG. 4. Nucleotide sequences of IS*51* (*A*), IS*52* (*B*), and their flanking regions. We show only sequences of the upper (5′→3′) strands, which is in the same direction of transcription of *iaaM*. The termini of the insert are indicated by the brackets. The terminal inverted repeats are underlined. ORFs found in the upper strands are indicated above the sequences: those of the opposite strands are shown below the sequence. The boxed sequences adjacent to the termini of the IS elements are the direct repeats of the target sites. Nucleotide sequences of the flanking region of IS*51* in pTET11 (TK2015-3) are shown on the top and those in pTET1 (PB205-1L) at the bottom of the figure in *B*. The dotted line represents putative ribosome binding site sequences (*A*).

IS*51* and IS*52* share features common to other IS elements found in prokaryotic organisms with respect to inverted repeats present at both ends, to a short duplication of target sequences at recombination sites (i.e., CAG for IS*51*, TTAG or CTAG for IS*52*), to the size, and to polar effects (19). As pointed out by Rak and Reutern (20) and others, many insertion elements have coding frames arranged in a characteristic manner: namely, a large ORF coding in one direction and a significantly shorter overlapping ORF in the opposite direction with an identical frame configuration (codons in phase). IS*51* has the same characteristics; however, IS*52* is somewhat unusual in that it has relatively large ORFs present on both strands (codons in phase).

The presence of a consensus sequence at the target sites is less common among IS elements already sequenced by others. Among the numerous prokaryotic IS elements characterized, IS*5* has been shown to have a consensus target sequence, CTAA, CAAG, or CTAG (21, 22). Although our data are based on samples from only three strains, it is interesting to note that both IS*51* and IS*52* are integrated after the consensus dinucleotide sequence of AG. There is, however, no consensus sequence at the right border. The

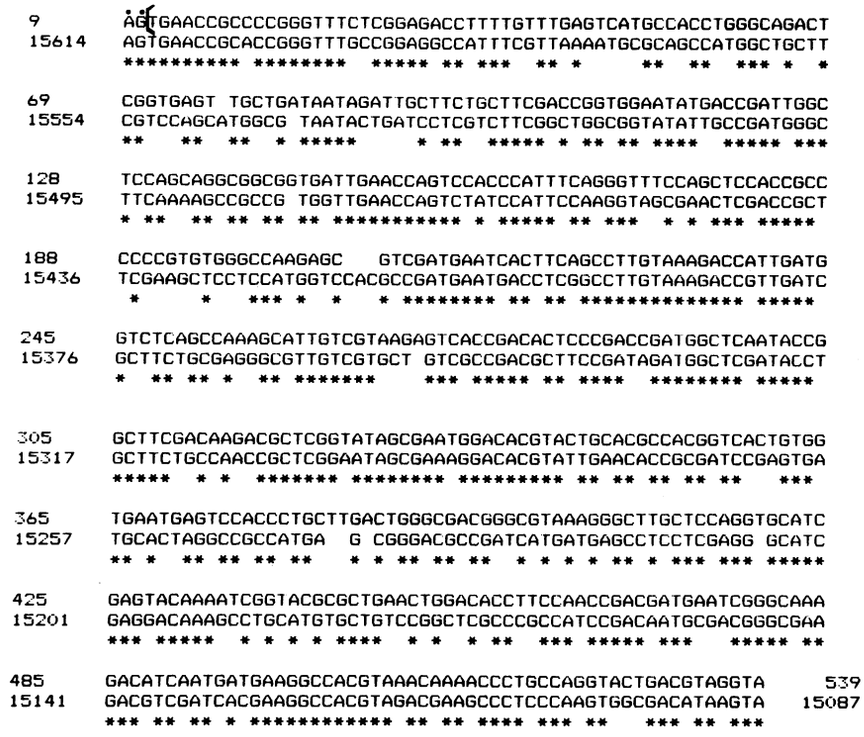

FIG. 5. Homology between the nucleotide sequences of IS*51* (first line) and the T_c region of T-DNA of *A. tumefaciens* (second line). One terminus of IS*51* is indicated by a bracket and a part of duplicated sequence, AG, at recombination junctions is dotted. Numbers on top refer to the nucleotide sequence of IS*51* in Fig. 4, and those on bottom refer to the nucleotide sequences of the T-DNA of octopine plasmid pTi15955 (18). Asterisks show the base matches.

dinucleotide pair AG also occurs at the left border of the sequences within the T_c region of T-DNA that show high homology with IS*51*. The two different repeated sequences in *nif* genes of *Rhizobium japonicum* also show the tetranucleotides sequence CTAG or GTAG at the recombination sites (23). Moreover, IS*51* terminates in the dinucleotide sequence TG–CA, which has also been observed in IS*3* (19), bacteriophage Mu, eukaryotic transposable elements, the provirus forms of retrovirus (24), and Tn*7* (25).

Earlier, we reported that *iaaM* and *iaaH* have significant homology with genes 1 and 2 of *A. tumefaciens* T-DNA, respectively (4). Suggestions have been made that the homology indicates a common origin for the genes or that genes 1 and 2 of T-DNA originated from *P. savastanoi iaaM* and *iaaH* (26). The homology observed between IS*51* and T_c region of *A. tumefaciens* T-DNA may indicate a remnant of an earlier transpositional event by which *iaaM* and *iaaH* were introduced into *A. tumefaciens* T-DNA from *P. savastanoi*. The copy of IS*51* that occurs 2.5 kb downstream from *iaaH* may represent one border of a transposon originally introduced into *A. tumefaciens* from *P. savastanoi*; the hypothetical *P. savastanoi* transposon that contained *iaaM* and *iaaH* subsequently underwent DNA rearrangement in *A. tumefaciens*, leaving only functionally active *iaaM* and *iaaH* conserved in the T-DNA. Interestingly enough, plasmids in several *Rhizobium* species have homologous sequences at or near the T_c region of T-DNA in the octopine type *A. tumefaciens* where IS*51* has a homology (27).

In the context of metabolic control, it will be interesting to determine if genes concerned with secondary metabolism in *P. savastanoi* are specific targets for inactivation by IS elements. Perhaps insertional inactivation of a gene for an enzyme diverting a primary metabolite into secondary metabolism may be a means of preventing a drain in primary metabolism when nutritional limitation becomes a threat to the survival of the bacterium.

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1. Comai, L. & Kosuge, T. (1982) *J. Bacteriol.* **149,** 40–46.
2. Kosuge, T., Heskett, M. G. & Wilson, E. E. (1966) *J. Biol. Chem.* **241,** 3738–3744.
3. Smidt, M. & Kosuge, T. (1978) *Physiol. Plant Pathol.* **13,** 203–214.
4. Yamada, T., Palm, C. J., Brooks, B. & Kosuge, T. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 6522–6526.
5. Thomashow, L. S., Leeves, S. & Thomashow, M. F. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 5071–5075.
6. Schroeder, G., Waffenschmidt, S., Weiler, E. W. & Schroeder, J. (1984) *Eur. J. Biochem.* **138,** 387–391.
7. Follin, A., Inze, D., Budar, F., Genetello, C., Van Montagu, M. & Schell, J. (1985) *Mol. Gen. Genet.* **201,** 178–185.
8. Van Onckelen, H., Prinsen, E., Inze, D., Rudelsheim, P., Van Lijsebettens, M., Follin, A., Schell, J., Van Montagu, M. & De Greef, J. (1986) *FEBS Lett.* **198,** 357–360.
9. Comai, L. & Kosuge, T. (1983) *J. Bacteriol.* **154,** 1162–1167.
10. Southern, E. M. (1975) *J. Mol. Biol.* **98,** 503–517.
11. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
12. Smith, H. O. (1980) *Methods Enzymol.* **65,** 371–389.
13. Messing, J. (1983) *Methods Enzymol.* **101,** 20–78.
14. Messing, J. & Vieira, J. (1982) *Gene* **19,** 269–276.
15. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
16. Biggin, M. D., Gibson, T. J. & Hong, G. F. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 3963–3965.
17. Bassel, B. A. (1979) *Nucleic Acids Res.* **6,** 2003–2016.
18. Barker, R. F., Idler, K. B., Thompson, D. V. & Kemp, J. B. (1983) *Plant Mol. Biol.* **2,** 335–350.
19. Iida, S., Meyer, J. & Arber, T. (1983) in *Mobile Genetic Elements*, ed. Shapiro, J. A. (Academic, New York), pp. 159–221.
20. Rak, B. & Reutern, B. V. (1984) *EMBO J.* **3,** 807–811.
21. Schoner, B. & Kahn, B. (1981) *Gene* **14,** 165–174.
22. Engler, J. A. & Van Bree, M. P. (1981) *Gene* **14,** 155–163.
23. Klauza, K., Harn, M. & Hennecke, H. (1985) *J. Bacteriol.* **162,** 535–542.
24. Temin, H. M. (1980) *Cell* **21,** 599–600.
25. Lichtenstein, C. & Brenner, S. (1982) *Nature (London)* **297,** 601–603.
26. Schell, J. (1985) in *Proceedings of the Stadler Genetic Symposia: Genetics, Development and Evolution*, ed. Gustafson, J. P. (Univ. of Missouri, Columbia), Vol. 16, in press.
27. Hadley, R. G. & Szalay, A. A. (1982) *Mol. Gen. Genet.* **188,** 361–369.