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Limited Host Range Ti Plasmids: Recent Origin from Wide Host Range Ti Plasmids and Involvement of a Novel IS Element, IS*868*

François Paulus, Jean Canaday, and Léon Otten

Department of Phytopathology, Plant Molecular Biology Institute, Strasbourg 67084, France.
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Agrobacterium tumefaciens **biotype III octopine strains have been isolated from grapevine tumors worldwide. They comprise limited and wide host range (LHR and WHR) strains that carry related tumor-inducing (Ti) plasmids with two T-regions, TA and TB. The WHR TA-region resembles the biotype I octopine region, whereas the LHR TA-region is a recent deletion derivative of the WHR TA-region, which lacks the *iaa* genes and part of the *ipt* gene. Sequencing of the TA-region of the ubiquitous LHR strain AB3 showed that the deleted region is replaced by an insertion sequence (IS) element, IS*868*, which resembles the IS*51* element of *Pseudomonas syringae* subsp. *savastanoi*. The Ti plasmid of LHR strain Ag57 carries essentially the same *iaa* gene deletion as pTiAB3, but lacks IS*868*. We propose that the LHR Ti plasmids arose by the recent insertion of an IS*868* element into the TA-region of a WHR-type Ti plasmid, followed by transposition to a nearby site. The deletion was caused during the second transposition or by later recombination between the two IS*868* copies. Biotype III octopine strains also carry an IS*51*-like sequence close to the TB *iaa* genes. Our results confirm and extend earlier observations indicating that IS*51*-like elements in *Pseudomonas* and *Agrobacterium* are associated with *iaa* genes and played a major role in Ti plasmid evolution.**

Additional keywords: plant tumorigenicity, bacterial insertion sequences.

Agrobacterium tumefaciens (Smith and Townsend) Conn induces tumors on susceptible plants by transferring one or two DNA segments (T-regions or T-DNAs) from a large plasmid (Ti plasmid) into the plant cells during infection. The transferred DNA is stably integrated into the nuclear DNA (Nester *et al.* 1984; Zambryski *et al.* 1989). Biotype I octopine and nopaline strains carry tryptophan-2-monooxygenase (*iaaM*), indoleacetamide hydrolase (*iaaH*), isopentenyl transferase (*ipt*), and 6b genes, which are expressed in the transformed plant cells and lead to tumor formation (Morris 1986; Tinland *et al.* 1989). Biotype III octopine strains are specifically associated with grapevine and have been isolated worldwide. These strains have been divided into limited and wide host range (LHR and WHR) strains according to their ability to induce tumors on tobacco (Thomashow *et al.* 1981; Knauf *et al.* 1982; Buchholz *et al.* 1984; Knauf *et al.* 1984; Yanofsky *et al.* 1985b). The TA-region of the biotype III octopine WHR strains strongly resembles the TL-region of the biotype I octopine WHR strains (Huss *et al.* 1989), whereas the TA-region of the LHR strains is a deleted version of the WHR TA-region (Knauf *et al.* 1984; Yanofsky *et al.* 1985b; Paulus *et al.* 1989a).

Although the T-DNA genes are expressed in eucaryotic cells, some of them are probably of procaryotic origin. *A. tumefaciens* (Beaty *et al.* 1986) and *Pseudomonas syringae* subsp. *savastanoi* (Powell and Morris 1986) carry bacterially expressed *trans*-zeatin secretion genes (*tzs*) with structural and functional resemblance to the *ipt* gene of the *Agrobacterium* T-region.

P. s. subsp. *savastanoi* (Yamada *et al.* 1985) and *Bradyrhizobium japonicum* (Sekine *et al.* 1989) carry *iaa* genes that are strongly homologous to the *iaa* genes of the *Agrobacterium* T-region. In *Pseudomonas*, the *iaa* genes are associated with the 1.3-kilobase (kb) bacterial insertion sequence (IS) element IS*51* and are present at different locations in different strains. This suggests that the *Pseudomonas iaa* genes are part of a mobile element (Yamada *et al.* 1986; Palm *et al.* 1989).

The 1.9-kb TC-region that is situated between the TL- and TR-regions of the biotype I octopine strain Ach5 (Barker *et al.* 1983) carries a 529-base pair (bp) fragment which is strongly homologous to one end of IS*51*, including the 26-bp inverted repeat sequence. This observation led to the proposal (Yamada *et al.* 1986) that *Agrobacterium* acquired its T-DNA *iaa* genes from another bacterial species through a transposition event involving IS*51*-like elements.

Here we describe an intact 1.3-kb insertion sequence (IS*868*) from the *Agrobacterium* biotype III LHR octopine strain AB3 that is homologous to IS*51* and is located within the TA-region. A detailed structural comparison of LHR and WHR TA-regions indicates that the biotype III octopine LHR Ti plasmids originated very recently from the biotype III octopine WHR Ti plasmids due to the transpositional activity of IS*868*.

Address reprint requests to L. Otten.

Nucleotide and/or amino acid sequence data is to be submitted to GenBank, EMBL, and DDBJ as accession number JO3692.

MATERIALS AND METHODS

Strains and plasmids. *Escherichia coli* NM522 (Gough and Murray 1983) and the phagemids pBluescript KS−

and KS+ (Stratagene, La Jolla, CA) were used for cloning and sequencing. *A. tumefaciens* biotype III strains AB3 (LHR), Tm4 (WHR, Szegedi 1985), and Ag57 (LHR, Knauf *et al.* 1982), the C58C9(pTiAg57) exconjugant LBA649 (Hoekema *et al.* 1984), and various other biotype III wild-type strains (Paulus *et al.* 1989a, 1989b) have been described previously.

Cloning and sequencing procedures. Cloning and sequencing procedures were as described by Sambrook *et al.* (1989). Sequences were analyzed with a MicroVAX using GCG software (Devereux *et al.* 1987).

DNA hybridization. DNA hybridization was conducted for 16 hr in 50% formamide, 4× SSC (1× SSC is 0.15 M NaCl, 0.015 M sodium citrate), 10× Denhardt's solution (1× is 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin), 0.2% sodium dodecyl sulfate, and

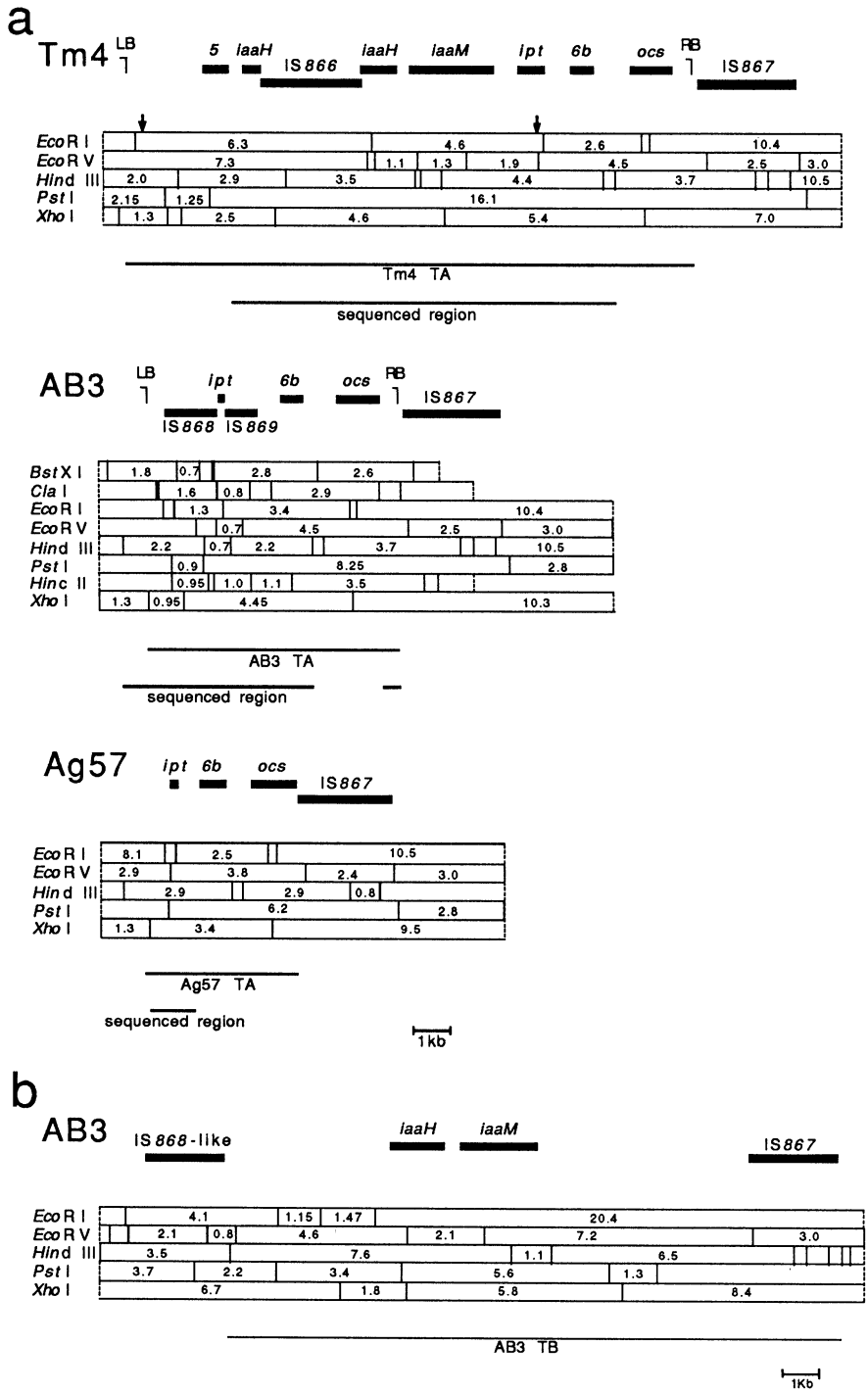

Fig. 1. **A**, TA-regions of limited host range strains AB3 and Ag57 and wide host range strain Tm4. **B**, TB-region of limited host range strain AB3. Mapped regions are delimited by dashed vertical lines. Arrows indicate the extent of the TA deletion in pTiAB3. LB and RB indicate left and right borders. The right TA border of Ag57 is absent (Paulus *et al.* 1991). The left TA border sequence of the Ag57 TA-region and the left and right border sequences of the AB3 TB-region were not identified by sequencing.

0.1 mg/ml calf thymus DNA at 42° C. Filters were washed with 2× SSC, 0.1% sodium dodecyl sulfate (three times for 15 min), and 2× SSC (once for 15 min) at room temperature and exposed for autoradiography.

RESULTS

The TA-region of pTiAB3. The general structure of the TA-region of pTiAB3 has been described previously (Paulus *et al.* 1989a). The pTiAB3 TA-region resembles the well-known TL-region of the biotype I octopine strains and the TA-region of the biotype III octopine strains like Tm4 (Huss *et al.* 1989; Paulus *et al.* 1989a), but the *iaa* genes and part of the *ipt* gene are deleted, as was also noted for the AB3-like strain Ag162 (Yanofsky *et al.* 1985a, 1985b). The TA maps of pTiAB3 and Tm4 are shown in Figure 1A.

Yanofsky *et al.* (1985a) noted that a 26-nucleotide sequence immediately to the left of the truncated *ipt* gene of the LHR strain Ag162 also occurred in the TC-region of pTiAch5. We found that this sequence corresponded to the inverted repeat of the IS*51*-like sequence discovered within the TC-region by Yamada *et al.* (1986). Subsequent homology studies using AB3 TA-region clones and Ach5 TC clones (F. Paulus, unpublished data) indicated that the AB3 TA-region might contain an intact IS*51*-like element. We therefore cloned and sequenced the corresponding region.

Cloning and sequencing of IS*868*. The 0.7-kb *Hin*dIII fragment and the two 2.2-kb *Hin*dIII fragments of the

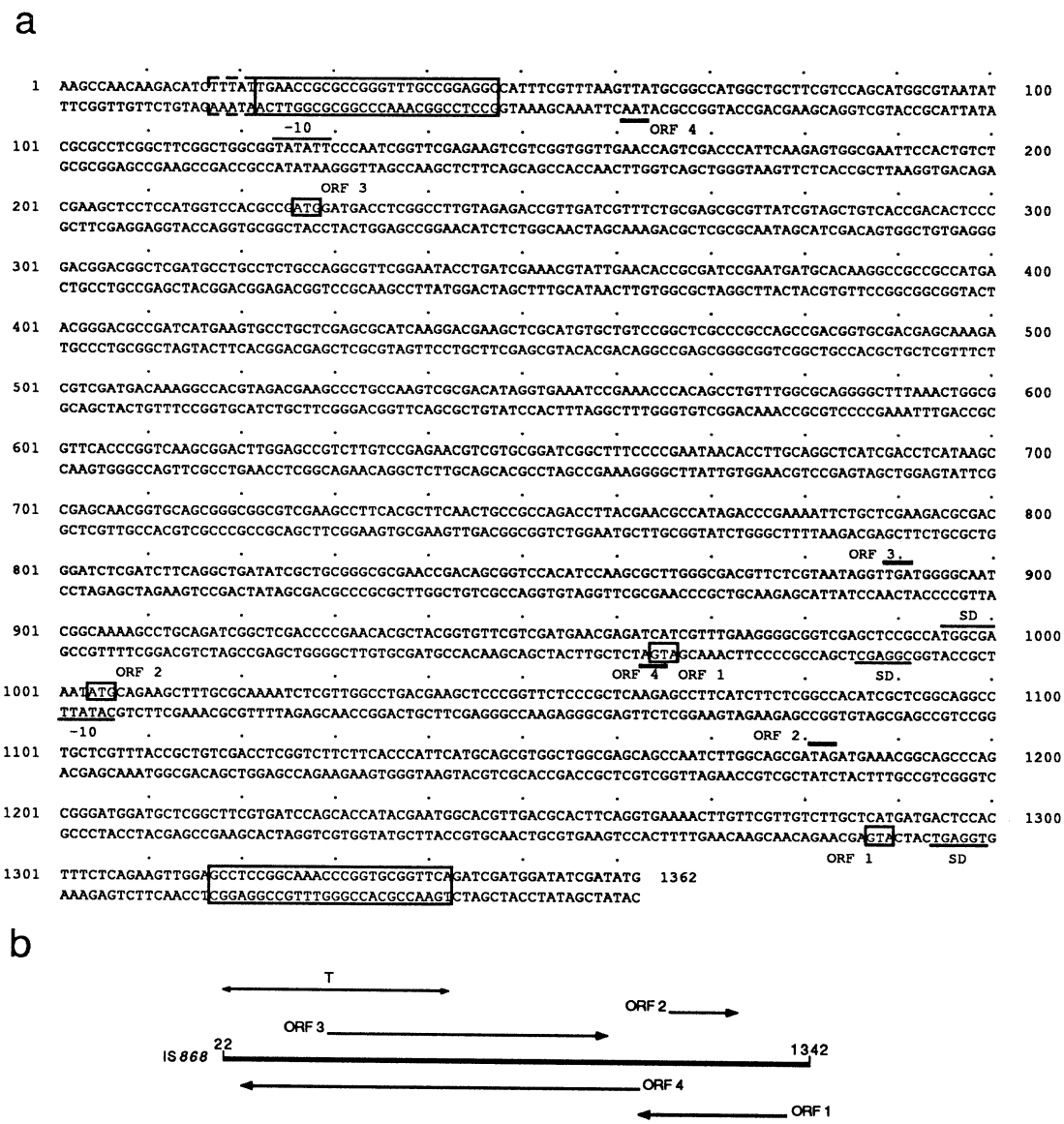

Fig. 2. A, Nucleotide sequence of IS*868* and part of the surrounding TA sequence. Small boxes indicate start codons; underlining, stop codons; large boxes, inverted repeats; dashed box, nucleotides missing in pTiAg57; and SD, Shine-Dalgarno sequence. −10 indicates the −10 consensus sequence. The gap algorithm of Needleman and Wunsch (Devereux *et al.* 1987) was used with the gap weight set at 5.00 and the length weight at 0.30. **B**, Open reading frame (ORF) organization of IS*868*. T designates the fragment conserved in the IS*51*-like fragment of pTiAch5 (Barker *et al.* 1983; Yamada *et al.* 1986); numbers indicate the nucleotide coordinates shown in **A**.

pTiAB3 TA-region (Fig. 1A) were cloned into the pBluescript KS− and KS+ vectors and sequenced. Coordinate 0 corresponds to the nucleotide situated at 1216 bp to the right of the *Hin*dIII site immediately to the left of the left TA border (584 bp to the left of this border). The end of the 5,151-bp sequence corresponds to the first *Hin*dIII site to the right of gene 6b. The left part of the TA-region sequence (from −1216 to 0) will be published elsewhere. From 22–1342 (EMBL accession number X55075), to the left of the truncated *ipt* gene, sequence analysis revealed a 1,321-bp insertion element (Fig. 2A), which is defined by 26-bp inverted repeats with one mismatch. This element, which we propose to call IS*868*, is 82% homologous to the truncated IS*51*-like sequence of the Ach5 TC-region (Barker *et al.* 1983; Yamada *et al.* 1986) and 65% homologous to IS*51* itself (Yamada *et al.* 1986). The right inverted repeat of IS*868* is identical to the repeat of the TC IS*51*-like sequence, and has 22 of 26 nucleotides in common with the IS*51* repeat. Immediately to the right of IS*868*, the truncated *ipt* gene is found (coordinates 1343–1556), followed by a sequence (from 1557–2407) that has been identified as an IS element, IS*869*, which is unrelated to IS*868* (Paulus *et al.*, in press). From 2407–3929, the AB3 TA sequences are again homologous to the TL-region sequence of pTiAch5 and 99.7% homologous to the sequence of the TA-region of the WHR strain Tm4 (Bonnard *et al.* 1989a; L. Otten, unpublished).

The IS*868* sequence has four putative open reading frames (ORFs), which are greater than 50 nucleotides (Fig. 2A, B), and displays various characteristic features of the IS*3* family (Schwartz *et al.* 1988; Galas and Chandler 1989): ORF 1 (which corresponds to ORF A of the IS*3* family) is followed by the large ORF 4, which is called ORF B in the IS*3* family where this ORF has been found to correspond to the transposase gene (Schwartz *et al.* 1988). The start codon of ORF 4 overlaps the stop codon of ORF 1 by two nucleotides. Fig. 3A and C show the predicted amino acid sequences for ORF 4 and ORF 1 of IS*868* and the corresponding ORFs of IS*51* (ORF B and ORF A, respectively). The amino acid sequence alignment of ORF 4 of IS*868* and ORF B of IS*51* can be considerably improved if the following two nucleotide changes are made in the IS*51* sequence (coordinates from Yamada *et al.* 1986): the addition of a C between position 657 and 658 and deletion of a G at position 418 (Fig. 3B). This also leads to a better match between ORF B of IS*51* and the corresponding ORF of the IS*3* family member IS*3411* (Ishiguro and Sato 1988). The IS*868* sequence has been checked thoroughly and is unambiguous. The amino acid sequence homologies between ORF 1 of IS*868* and the corresponding ORFs of the well-characterized elements IS*51*, IS*3411*, and IS*3* (Schwartz *et al.* 1988; Galas and Chandler 1989) are 64.8, 57.4, and 26.7%, respectively; for ORF 4, these values are 55.2, 50.8, and 28.8%.

IS*868* is not flanked by a direct 3-bp repeat (Fig. 2A), which is contrary to IS*51* (Yamada *et al.* 1986) and most other members of the IS*3* family (Galas and Chandler 1989).

Occurrence of IS*868* and IS*868*-related sequences in AB3

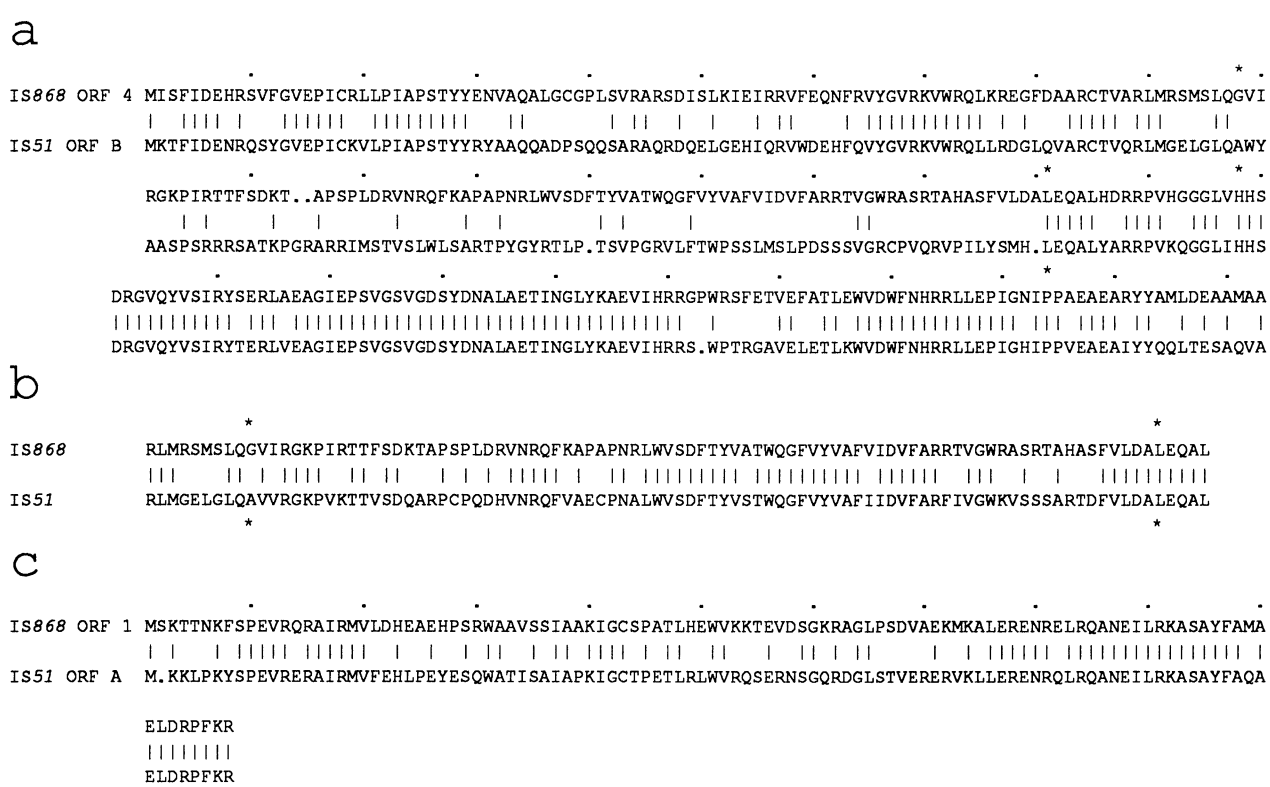

Fig. 3. A, Predicted amino acid sequences of open reading frame (ORF) 4 of IS*868* and the corresponding predicted amino acid sequences of ORF B of IS*51* (Yamada *et al.* 1986). The stars indicate the fragment shown in **B**. **B**, Improved alignment of ORF 4 of IS*868* and ORF B of IS*51* after the addition of a C between positions 657 and 658 (Yamada *et al.* 1986) and deletion of a G at position 418. Only the fragment between the stars indicated in **A** is shown. **C**, Predicted amino acid sequence of ORF 1 of IS*868* and ORF A of IS*51*.

and other biotype III strains. To test for the possible presence of IS*868* at other locations in the AB3 genome, the internal 950-bp *Hinc*II IS*868* fragment (Fig. 1A) was hybridized against *Hin*dIII- or *Hinc*II-digested total and plasmid DNA of AB3. The IS*868* probe hybridized strongly to the TA element and more weakly to a fragment (not shown) that was mapped by using various pTiAB3 clones (F. Paulus, unpublished data) to the left of the pTiAB3 TB-region at 4.5 kb from the TB-*iaaH* gene (Fig. 1B). The IS*868* probe was also hybridized to total DNA and plasmid DNA of other biotype III strains described previously (Paulus *et al.* 1989a, 1989b). Various fragments hybridizing to IS*868* were identified (Table 1). The TA restriction fragments typical of the TA IS*868* element were found in all 17 AB3-like strains and showed the expected strong hybridization. Remarkably, the LHR strain Ag57 did not show strongly homologous sequences, although preliminary studies (F. Paulus, unpublished data) indicated that pTiAg57 has a large TA deletion like pTiAB3 (see Fig. 1A and below). The Tm4-like strains and the nopaline strains showed one or more weak bands. All biotype III octopine strains carried the previously noted IS*868*-like sequence close to the TB-*iaaH* gene. This sequence is characterized by a 3.5-kb *Hin*dIII fragment (4.5 kb in Tm4, Huss *et al.* 1989) and a 2.0-kb *Hinc*II fragment. Total DNA of octopine and nopaline strains showed additional, very weakly hybridizing bands that were not further investigated. Finally, the IS*868* probe strongly hybridized to the Ti plasmids of the vitopine strains S4, Sz1, and Sz2 and to an S4 strain plasmid of unknown function.

Table 1. Fragments hybridizing to IS*868* in plasmid and chromosomal DNA of different biotype III strains

Designation[a]	*Hin*dIII fragments[b] (kilobases)	*Hinc*II fragments[b] (kilobases)
Octopine strains with a small TA-region		
AB3 (Zw2, B10/7, AT6, 2612, 2613, 2614, 2644, 2650, 2651, 2653, 2654, 2655, 2656, 2675, 2676, 2677)	*0.7*, *2.2*, 3.5 (22.9), (4.1), (5.0), (6.2), (10.5)	*0.95*, 2.0 (3.0), (6.1), (7.2)
Ag57-LBA649	3.5 (2.9), (4.1), (5.0), (10.5)	2.0 (3.0), (3.1), (7.2)
Octopine strains with a large TA-region		
Tm4	4.5	2.0
K305 (K308, 2649, 2678, 2679, 2680, 2686)	**2.4**, **2.6**, 3.5	**1.5**, 2.0
2618 (2608, 2615, 2645, 2646, 2647)	**2.4**, 3.5, **7.2**	**1.5**, 2.0, **2.2**
Hm1	**2.1**, 3.5, **10.5**	2.0, **2.2**
Vitopine strains		
S4	3.7, *7.8*, *10.5*, *> 12.0*	0.4, *0.5*, *0.9*, *1.5*
(Sz1, Sz2)	3.7, *7.8*	Not tested
Nopaline strains (2609, AT1, IS1.1, EK2, 643)	7.6[c]	Not tested

[a] Strains between parentheses were only analyzed by *Hin*dIII digests of total DNA.
[b] Lengths of plasmid sequences are given in normal type, and lengths of chromosomal sequences are in bold type; fragments showing strong hybridization are indicated in italic type, and fragments showing very weak hybridization are given between parentheses.
[c] Only determined for total DNA.

The TA-region of pTiAg57. Since the TA-region of pTiAg57 from the LHR strain Ag57 unexpectedly lacked most or all of the IS*868* sequences, pTiAg57 was studied in more detail. pTiAg57 has been described as an LHR octopine Ti plasmid with host range properties similar to those of pTiAg162 and pTiAB3 (Knauf *et al.* 1982; Hoekema *et al.* 1984). Preliminary studies showed that the restriction map of the pTiAg57 TA-region differed from the pTiAB3 map, and that the Ag57 strain was the only such strain in our collection. We therefore cloned the TA-region of pTiAg57 and established a homology map with pTiAch5 probes (Fig. 1A). The 2.9-kb *Hin*dIII TA fragment was cloned in both orientations in the pBluescript KS− vector, and a 1,156-nucleotide region between the corresponding AB3 coordinates −476 and 2856 was sequenced. Starting from the left, both sequences are identical from −476 to 16 (not shown). The sequences found in AB3 at position 17–1343 are missing in Ag57. The AB3 and Ag57 sequences are again identical from position 1343 to 1556. Ag57 lacks the sequences found at position 1557–2407 in AB3 that correspond to the AB3-specific IS element IS*869* (Paulus *et al.*, in press) and which include its 4-bp insertion site duplication. From position 2408 to 2856, the AB3 and Ag57 sequences are again identical. Thus, the AB3 and Ag57 TA-regions show a very similar TA-region deletion, but in Ag57 the TA deletion extends five nucleotides to the left and the IS*868* element is absent. The common regions are 100% homologous.

DISCUSSION

Yamada *et al.* (1985) discovered that the *iaa* genes in *P. s.* subsp. *savastanoi* and *A. tumefaciens* shared homology. Moreover, they noted that a 529-bp fragment in the 1.9-kb TC-region between the TL- and TR-regions of pTiAch5 strongly resembled the left end of the 1,311-bp IS*51* element associated with the *Pseudomonas iaa* genes (Yamada *et al.* 1986). They proposed, therefore, that the *Agrobacterium* T-DNA *iaa* genes may have been acquired from other bacterial species as part of a transposon flanked by IS*51*-like elements (Yamada *et al.* 1986; Palm *et al.* 1989). The IS*51*-like sequence of pTiAch5 (indicated by T in Fig. 2B) is incomplete, and no related Ti plasmids have been described that lack the sequence or carry variants of it. Thus, it is not possible to retrace the evolutionary history of this element.

The studies reported here show that an apparently complete 1,321-bp IS*51*-like element is present in the TA-region of the biotype III LHR strain AB3 as well as in all 16 natural isolates of the AB3 type investigated so far. We propose to call this element IS*868*. IS*868* replaces the deleted TA-region fragment and is not found in WHR strains. IS*868* is related most closely to the IS*51*-like sequence of the Ach5 TC-region (82% nucleotide sequence homology) but also shares significant homology with the following IS elements of the IS*3* family (Schwartz *et al.* 1988; Galas and Chandler 1989): IS*629* (*Shigella sonnei* (Levine) Weldin, Matsutani *et al.* 1987, only two fragments sequenced, 61.6% overall homology), IS*3411* (*E. coli*, Ishiguro and Sato 1988, 60.3%), and IS*6110*

(*Mycobacterium tuberculosis* (Zopf) Lehmann and Neumann, Thierry *et al.* 1990, 59.8%). On the basis of its resemblance to the members of the IS*3* family, IS*868* should have generated a 3-bp target site duplication (Yamada *et al.* 1986; Schwartz *et al.* 1988; Galas and Chandler 1989). No such duplication was found. Although the sequences could have diverged since the insertion event, this seems unlikely because the remaining Tm4 and AB3 sequences are identical. Even if divergence has occurred, it is difficult to imagine how the IS*868* element could have been inserted at the precise site of an earlier deletion. We propose, therefore, that the IS*868* element of the AB3 TA-region resulted from two IS*868* insertions within the TA-region in the direct orientation, one within the *ipt* gene and the other 2.8 kb to the left of the TA-*iaaH* gene. The second transposition led to recombination between the two IS*868* copies (either during transposition or after it) and deletion of the internal, 5.5-kb region with its two *iaa* genes and part of the *ipt* gene, giving rise to the typical TA-region structure of the LHR strains.

Intramolecular recombination between two identical IS elements and loss of the internal region have been described for the IS*868*-related IS*3411* element, two copies of which flank the citrate utilization genes in *E. coli* (Ishiguro and Sato 1988). Note that in this model, the TA-*iaa* genes were temporarily flanked by IS*868* elements and were, therefore, part of a transposon-like structure (IS*868*-*iaa*-IS*868*). It may be asked whether the octopine TA-region could have acquired its *iaa* genes through insertion of this transposon into a primitive, *iaa*-less TA-region, in line with the hypothesis of Yamada *et al.* (1985). However, the interruption of the *ipt* gene clearly shows that the TA-*iaa* genes were present before the transposon was formed. Moreover, the Tm4 TA-region carries no traces of IS*868* elements, and neither does the related Ach5 TL-region. However, the association of IS*51*-like elements and *iaa* genes, first noted by Yamada *et al.* (1986) for *Pseudomonas* and an *Agrobacterium* biotype I strain, is further illustrated here by the IS*868* element, which was inserted close to the TA-*iaa* genes, and the occurrence of an IS*51*-like element 4.5 kb to the left of the TB-*iaaH* gene. The reasons for these associations remain unknown.

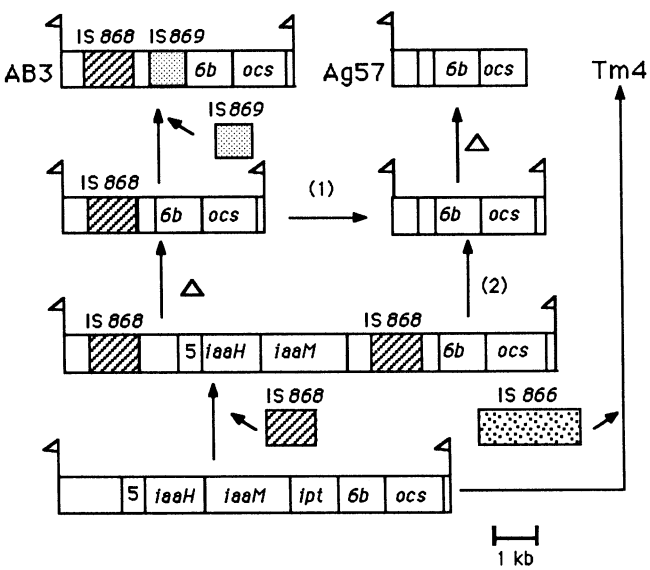

Fig. 4. Proposed evolution of the TA-region of octopine Ti plasmids. (1) and (2) indicate two possible excision pathways. Δ designates deletion. Flags indicate T-DNA borders. For further details, see text.

Most likely, the insertion of the two IS*868* elements occurred in a WHR octopine Ti plasmid that did not yet carry the IS*866* element in the TA-*iaaH* gene. This assumption is based on the fact that LHR strains do not contain the IS*866* element, whereas most WHR strains carry one Ti plasmid copy and several chromosomal copies; this suggests that the first IS*866* element which entered the WHR strains was inserted into the TA-region and then rapidly generated further chromosomal copies (Paulus *et al.* 1989b).

The deletion of the internal TA-region of the putative WHR ancestor of pTiAB3 eliminated three oncogenes and may have changed the tumor induction properties of the resulting strain on grapevine. However, infection studies with LHR and WHR strains on grapevine (F. Paulus, unpublished) showed both to be equally oncogenic, and studies with various T-region mutants of the WHR strain Tm4 (Huss *et al.* 1990) showed that the deletion of the TA-*iaa* and *ipt* genes had no consequence for tumor induction on grapevine. Possibly, the oncogenic composition of the WHR-like ancestor strain of AB3 (two sets of *iaa* genes, an *ipt* gene, and gene 6b) resulted from selection for efficient tumor formation on another host plant. The AB3 ancestor may have become efficient on grapevine due to some other property. The TA structures of AB3 and Ag57 would then result from elimination of nonessential regions by the fortuitous insertion of IS elements.

Although pTiAg57 lacks the IS*868* element, its TA-region deletion is very similar to the pTiAB3 TA-region deletion: the right end of the deletion is the same, and the left end extends five more nucleotides to the left. This indicates that the pTiAg57 TA-region has been derived from the same intermediate IS*868*-*iaa*-IS*868* TA-region structure as proposed for pTiAB3. In the case of pTiAg57, the complete transposon may have been excised, or the Ag57 TA-region may have been derived from the AB3 TA-region by excision of the IS*868* element. Either one of these events might also be responsible for the additional deletion of five nucleotides.

Apart from IS*868*, we have detected several other unrelated IS elements in the biotype III octopine Ti plasmids. IS*867*, which is present in all biotype III octopine strains (Fig. 1A, B), probably arrived shortly before the LHR-WHR divergence, as indicated by the fact that all LHR and WHR Ti plasmids carry two characteristic IS*867* copies but show variable numbers of chromosomal IS*867* copies at locations which are different for LHR and WHR strains (Paulus *et al.* 1989b). The TA-*iaaH* gene of WHR strains is interrupted by an IS*866* element, which is not found in LHR strains (and is not found in the biotype I octopine Ti plasmid) and, therefore, presumably appeared after the LHR-WHR divergence (Huss *et al.* 1989). Finally, the AB3 TA-region contains the IS*869* element, which is situated to the immediate right of the truncated *ipt* gene and is absent from the WHR strains and the LHR strain

Ag57, indicating its arrival after the AB3-Ag57 divergence (Paulus *et al.*, in press). The various insertion and deletion events in the TA-regions of LHR and WHR strains occurred very recently, as is shown by the exceptional conservation of the AB3, Ag57, and Tm4 TA sequences. Despite this, both LHR and WHR strains occur worldwide (Paulus *et al.* 1989a). Since biotype III strains have been found mainly on grapevine, we suggest that these strains recently became associated with *Vitis vinifera* L., possibly when large-scale cultivation of this plant started. The observed differences in TA structure may then be due to adaptation to the new host. It is particularly interesting to note that IS elements, rather than nucleotide changes, generated the different strain types; this suggests that IS elements are much more efficient in generating genetic diversity. At least in the case of IS*866* (Bonnard *et al.* 1989b), IS*868* (this study), and IS*869* (Paulus *et al.*, in press), the T-region IS elements do not seem to be derived from endogenous elements, because they are not found in other *Agrobacterium* strains with closely related Ti plasmids. This suggests another possible explanation for the observed T-region diversity: the structural changes may be due to the accidental "infection" of the initial grapevine-associated *Agrobacterium* strains with IS elements from other grapevine-associated bacteria without necessarily generating selective advantages. In the latter case, the survival of the observed structures may be due to their coselection with other, unidentified traits. It remains to be investigated how the different strain types and their distributions reflect adaptations to different grapevine varieties or to different environments. The evolutionary events that we propose to have led to the emergence of the present-day LHR and WHR octopine strains are summarized in Figure 4. The other IS elements that have been detected in *Agrobacterium* are as follows: IS*60* (Ooms *et al.* 1981), IS*66* (Machida *et al.* 1984), IS*426* (Vanderleyden *et al.* 1986), and IS*427* (De Meirsman *et al.* 1989). However, the frequency of occurrence of these IS-containing strains was not studied. This makes it difficult to propose an evolutionary role (if any) for these other elements.

The vitopine strains (Szegedi 1985; Paulus *et al.* 1989a) also carry pTi-associated IS*868*-like elements. Vitopine strains show no homology to *iaa* genes, and their T-regions have not yet been identified. Further studies are required to establish the possible role of these IS*868*-like sequences in vitopine pTi evolution. Finally, the resemblance between IS*868* and a number of insertion elements that are found in very different bacterial species points to the possibility of horizontal gene transfer between *Agrobacterium* and other, unrelated bacteria.

ACKNOWLEDGMENTS

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