JOURNAL OF BACTERIOLOGY, Oct. 1986, p. 237–243
0021-9193/86/100237-07$02.00/0

Vol. 168, No. 1

Location of the Right Boundary of the Virulence Region on *Agrobacterium tumefaciens* Plasmid pTiC58 and a Host-Specifying Gene Next to the Boundary

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Received 18 February 1986/Accepted 19 June 1986

The right boundary of the virulence (Vir) region of the nopaline plasmid pTiC58 of *Agrobacterium tumefaciens* was determined by transposon insertion, cartridge emplacement, and deletion mutagenesis. Genetic complementation with mutant and wild-type alleles led to the identification of the *virE* locus at the right boundary, which was located about 6 kilobases from the left border of the segment of DNA that is transferred into the plant genome. *virE* is 2.0 kilobases long and encodes at least one protein of 69 kilodaltons. Various mutations in *virE* resulted in different truncated lengths of the 69-kilodalton protein. As this protein was increasingly truncated from the carboxy terminus, the host range of *A. tumefaciens* and the frequency of tumor formation diminished concomitantly. Thus, as one of its functions, the 69-kilodalton protein of *virE* is probably involved in some aspect of the host range specificity of *A. tumefaciens* and in infection efficiency.

Neoplastic tumors in plants characterized as crown gall are induced by *Agrobacterium tumefaciens*. The genes responsible for tumorigenesis are contained in a 200-kilobase (kb) tumor-inducing (Ti) plasmid (32, 33, 35). Genetic studies of the Ti plasmid have revealed two important sectors, the T and Vir regions, which contribute directly to the process of tumor formation (11, 14, 26). The T region DNA is transferred to plant cells and integrated in the nuclear DNA, resulting in tumor formation (3). Unlike the T region, the Vir region is not stably maintained in transformed plant cells but is absolutely essential for virulence (3, 12, 16, 17, 20, 22, 24). The Vir region has been genetically defined by transposon insertion mutagenesis (6, 15, 16, 24, 26). Although specific functions of the *vir* genes have not been established, these genes may provide all the functions necessary for the processing and transfer of the DNA of the T region from the bacterial cell to the plant cell. The Vir regions of octopine and nopaline Ti plasmids and of root-inducing plasmids share DNA sequence and functional homology (8, 9, 13, 16, 27, 34), but the left and right boundaries have not been precisely defined. Within the Vir region of pTiC58, at least six essential complementation groups have been identified (24), and one of them, identified as the host-dependent-variation (*hdv*) operon (*virD*), encodes proteins of 15, 56, and 28 kilodaltons (kDa) (11) and probably two additional proteins in *Agrobacterium* species (unpublished data). A second locus identified as *virC1* (formerly *bakA*) encodes a 25.6-kDa protein that is immunologically related to the *virC1* protein of octopine Ti plasmids (T. J. Close, R. C. Tait, T. Hirooka, L. Kim, and C. I. Kado, submitted for publication). Like pTiC58, the octopine Ti plasmids pTiA6NC and pTiAch5 contain complementation groups, *virA*, *B*, *C*, *D*, *E*, and *G*, which are also essential for virulence (15–17, 22).

Presented in this communication is evidence for the location of the right boundary of the Vir region of pTiC58 and for a host-range-specifying gene, *virE*.

(A portion of this work was presented at the First International Congress of Plant Molecular Biology, abstr. p63, 27 October 1985, Savannah, Ga.)

MATERIALS AND METHODS

Bacterial strains, plasmids, and media. The Rec⁻ pTi-free strain LBA4301 (21) and the virulent strain NT1 (pTiC58 Tra^c) (24) of *A. tumefaciens* were grown in medium 523 (18) at 30°C. *Escherichia coli* HB101 (2) was grown in LB medium (25). Filter-sterilized antibiotics (Sigma Chemical Co., St. Louis, Mo.) were added to the media at the following concentrations (in micrograms per milliliter): for *A. tumefaciens*: rifampin, 25; neomycin, 50; kanamycin, 50; tetracycline, 5; spectinomycin, 50; chloramphenicol, 3; and for *E. coli*: ampicillin, 50; neomycin, 50; kanamycin, 50; spectinomycin, 50; chloramphenicol, 20. The characteristics of cloning vectors pSa4, pUCD2, pUCD4, and pUCD9p (5, 31) and the recombinant plasmids are described in Table 1.

Plasmid DNA preparation. The procedures for preparative plasmid DNA isolation from *A. tumefaciens* (20) and *E. coli* (1) and rapid small-scale analytical methods for plasmids in *A. tumefaciens* (19) and *E. coli* (4) have been described.

Transformation. Transformation was performed for *E. coli* (4) and for *A. tumefaciens* (20) as described previously.

Enzymes. Restriction endonucleases were purchased from New England Biolabs, Beverly, Mass. T4 DNA ligase was purified by R. C. Tait. The nucleolytic cleavage and ligation reaction conditions were those recommended by the suppliers.

Cloning of pTiC58 DNA. pTiC58 Tra^c DNA was digested partially with *Xba*I and cloned in pUCD4. The DNA was digested with *Kpn*I and cloned in pUCD2 (Table 1).

Tn*5* mutagenesis. pTH60 contains a 30.3-kb fragment (*Xba*I fragments 4 and 5 of pTiC58 Tra^c) cloned in pUCD4 (Table 1). The recombinant plasmid was mutagenized in *E. coli* with phage lambda (*b*221, *O*am80, *P*am29, rex::Tn*5*,

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TABLE 1. Recombinant plasmids used in this study[a]

Plasmid	Cloned pTiC58 fragment(s)	Size of cloned fragment (kb)	Vector	Phenotype	Reference or source
pUCD2	None			Ap^r Tc^r Km^r Gm^r Sp^r	5
pUCD4	None			Ap^r Tc^r Km^r Gm^r Sp^r	5
pUCD9p	None			Ap^r Km^r Gm^r	5
pSa4	None			Cm^r Km^r Gm^r	31
pTH60	*Xba*I fragments 4 and 5	29.2	pUCD4	Ap^r Tc^r Km^r Gm^r Sp^r	This paper
pTH202	*Xba*I fragment 4	15.8	pUCD4	Ap^r Tc^r Km^r Gm^r Sp^r	This paper
pUCD2-K9$_1$	*Kpn*I fragment 9	8.0	pUCD2	Ap^r Tc^r	T. J. Close
pUCD2-K9$_2$	*Kpn*I fragment 9 (opposite orientation)	8.0	pUCD2	Ap^r Tc^r	T. J. Close
pUCD9p-K9$_2$	*Kpn*I fragment 9	8.0	pUCD9p	Ap^r	This paper
pA16	*Kpn*I fragment 9	8.0	pSa4	Cm^r	R. C. Lundquist
pTH303	*Kpn*I fragment 9, CTN	10.2	pUCD2	Ap^r Tc^r Nm^r	This paper
pTH60-57	*Xba*I fragments 4 and 5, Tn*5*	35.3	pUCD4	Ap^r Tc^r Km^r Gm^r Sp^r Nm^r	This paper
pTH60-59	*Xba*I fragments 4 and 5, Tn*5*	35.3	pUCD4	Ap^r Tc^r Km^r Gm^r Sp^r Nm^r	This paper

[a] Tn*5* and CTN insertion sites in pTH303, pTH60-57, and pTH60-59 are shown in Fig. 6.

*c*I857) (24). The resulting plasmids carrying transposon Tn*5* insertions were characterized by restriction mapping, and those containing Tn*5* insertions in the 30.3-kb fragment were transferred into recombination-proficient *A. tumefaciens* NT1 harboring pTiC58 Tra^c, in which reciprocal recombination (30) of the fragment was promoted as described previously (24). The recombinant Ti plasmids were transferred conjugatively to strain LBA4301, followed by selection for resistance to Rm and Nm and screening for sensitivity to Tc and UV light. Restriction analysis was used to verify that the 30.3-kb fragment had indeed recombined with pTiC58 Tra^c as described previously (24).

Cartridge insertion. A *cat-nptII* (CTN) cartridge constructed by T. J. Close contained a promoterless gene for chloramphenicol acetyltransferase (*cat*) from Tn*9* and a promoter-proficient neomycin phosphotransferase (nptII) gene (29) from Tn*5* (Fig. 1). The CTN cartridge was used to identify promoter-active fragments. The CTN cartridge was inserted into a *Bam*HI site of *Kpn*I fragment 9 of pTiC58 that was cloned in pUCD2-K9$_2$ (Table 1). The 3.3-kb *Hin*dIII fragment of Tn*5* including the *nptII* gene (*nptII* cartridge) was inserted into a unique *Hin*dIII site of *Kpn*I fragment 9 of pTiC58 cloned in pA16 by R. A. Lundquist (Table 1). These cartridge emplacement-mutagenized fragments were subsequently introduced into pTiC58 Tra^c by reciprocal recombination (24, 30).

Deletion. A deletion plasmid (pTH403) was constructed by digesting pTH202 (Table 1), containing *Xba*I fragment 4 in pUCD4, with *Bam*HI and *Bgl*II, followed by ligation with *Bam*HI fragment 15, which resulted in the deletion of a 3.2-kb internal fragment. A CTN cartridge was inserted into the site of the deletion (Fig. 2), and the mutagenized fragment was recombined with pTiC58 Tra^c as described above.

Virulence assay. Virulence assays of *A. tumefaciens* were performed on the stems of sunflower, tomato, potato, jimson weed, bachelor button, and *Vinca minor*, on the crown of lettuce, and on the upper leaves of *Kalanchoë daigremontiana*. Inocula grown on 523 agar medium for 2 to 3 days were placed on stems, crowns, or leaves and stabbed with sterile toothpicks into the respective tissues. Plants were grown in an air-conditioned greenhouse and scored for visible tumors after 8 to 10 weeks. The frequency of tumor formation was defined as the number of inoculation sites forming tumors versus the total number of sites inoculated. Small nondisruptive swellings that might occur at or near inoculated sites were not scored.

Construction of merodiploid strains. The recombination-deficient *A. tumefaciens* strain LBA4301, harboring pTiC58::Tn*5* or pTiC58::CTN mutant plasmids (phenotypically Rm^r and Nm^r), was used for constructing all merodiploids. The complementing plasmid, which contained the corresponding wild-type DNA fragment (or a DNA fragment with another mutation) carried by the vector pUCD2 or pUCD4 (Table 1), was introduced into these strains by transformation. The merodiploid construction was confirmed by the Rm^r Nm^r Tc^r phenotype and by plasmid DNA analysis (19). The complementing plasmids used in this study are listed in Table 1.

Analysis of proteins. The minicell-producing *E. coli* strain AS1522, kindly provided by A. O. Summers, was transformed with the plasmids of interest. That pTiC58-encoded proteins in *A. tumefaciens* are identical to those produced in *E. coli* minicells has been shown previously (12). The minicells were purified, and the plasmid-encoded proteins were labeled with [^{35}S]methionine (specific activity, 1,100 Ci/mmol; Amersham Corp.) by the method of Roozen et al. (28). Proteins were resolved electrophoretically as described

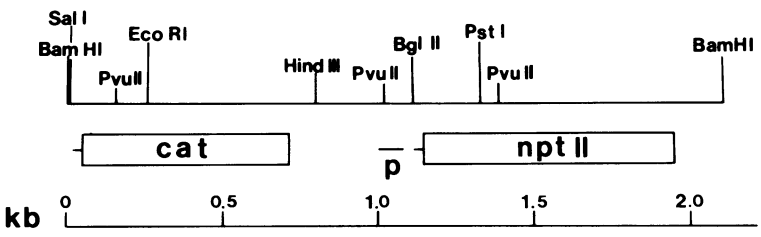

FIG. 1. Restriction map of CTN cartridge showing regions of the *cat* and *nptII* genes. p, Native promoter.

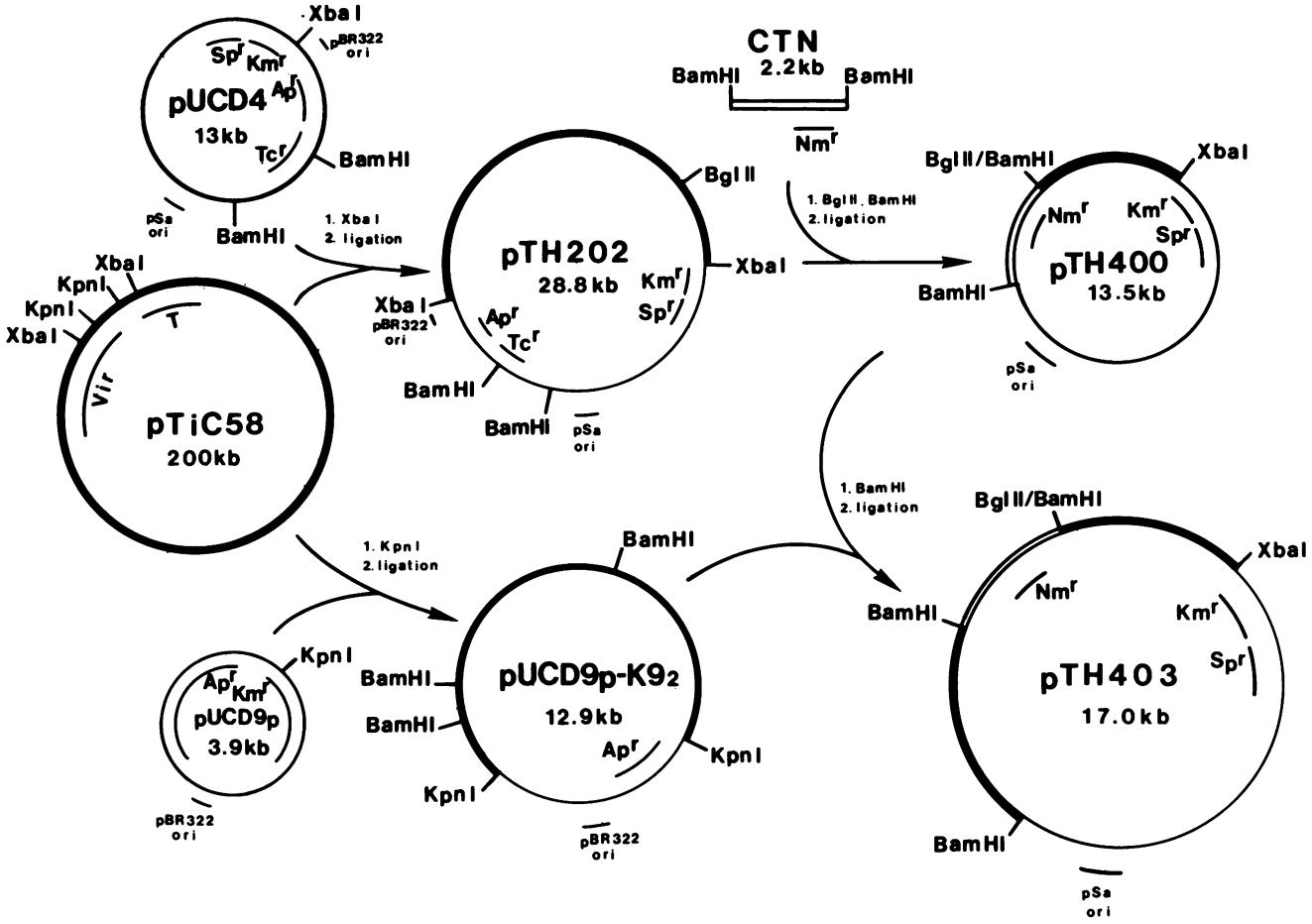

FIG. 2. Construction of pTH403. pTiC58*Xba*I fragment 4 and *Kpn*I fragment 9 were cloned in pUCD4 and pUCD9p, which resulted in pTH202 and pUCD9p-K92, respectively. pTH202 digested with *Bgl*II and *Bam*HI and ligated to the CTN cartridge gave rise to pTH400. pTH403 was made by inserting *Bam*HI fragment 15 (Fig. 4) (6) in pUCD9p-K92 into a unique *Bam*HI site in pTH400.

previously (11). ^{14}C-labeled molecular weight standards for the electrophoretograms were obtained from Amersham.

RESULTS

Determination of the right boundary of the Vir region. Tn*5* insertion, cartridge emplacement, and deletion mutants were constructed to determine the right boundary (the boundary nearest the T-DNA) of the Vir region. pTH60, comprising the right half of the Vir region and the left border of the T region of pTiC58 as *Xba*I fragments 4 and 5 (Fig. 3), was used as the target DNA for mutagenesis. pUCD2-K9$_2$ and pA16 containing CTN and *nptII* cartridges were also used. In addition to Tn*5* and cartridge insertion mutants, a 3.2-kb deletion, JK522, was introduced to test a 2.7-kb gap between coordinates 6.3 and 9.0 kb (Fig. 4) that contained no Tn*5* insertional mutation sites. pTH60, containing the putative right boundary region, which carried each of 16 mutations in the right half of the *Xba*I insert (Fig. 4), was introduced into wild-type pTiC58 DNA by reciprocal recombination (see Materials and Methods). The pTiC58 mutants obtained were verified by restriction mapping and tested for virulence. Mutants JK518 and JK519, JK505, JK521, and JK506 were avirulent on some host plants but weakly virulent on others (Table 2). Mutants with insertions located to the right of insertion JK507 were fully virulent, like wild-type pTiC58 (Fig. 4; Table 2). These results suggested that the right boundary of the Vir region is between the Tn*5* insertion sites of JK506 and JK507 and is located about 6 kb from the left border of T-DNA. JK516 and JK517 were completely virulent and thought to contain mutations mapping between *virD* and *virE*. Mutations to the left of JK516 (or JK517) were avirulent (Table 2).

Complementation analysis. Complementations were carried out by constructing merodiploid strains and assaying them for virulence on tomato, sunflower, and kalanchoë (Table 3). The complementing plasmids were as follows: pUCD2-K9$_1$ and pUCD2-K9$_2$, containing *Kpn*I fragment 9 of pTiC58 in pUCD2 in both orientations; pTH303, containing *Kpn*I fragment 9 with a CTN cartridge in a *Bam*HI site (the same site as JK518); and pTH60-57 and pTH60-59, containing *Xba*I fragments 4 and 5 with a Tn*5* inserted at the same mutation sites as JK505 and JK506, respectively (Fig. 5). The virulence of mutants JK518, JK505, and JK506 was completely restored (as characterized by fast-growing tumors) when complemented with wild-type *Kpn*I fragment 9 in *trans*. However, virulence was not restored in any of the mutants when complemented with the mutagenized fragment pTH303 or pTH60-57. pTH60-59 restored the virulence of JK518 and JK505, but was characterized by slow-growing tumors. Complementation of JK506 did not alter its original weakly virulent phenotype (Table 3). These results suggest

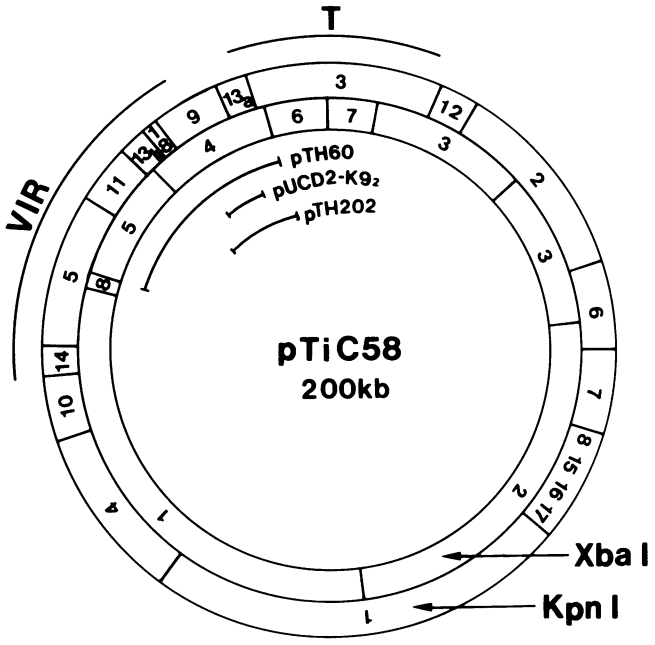

FIG. 3. *Kpn*I and *Xba*I restriction map of pTiC58 (6) showing regions included in the clones pTH60, pUCD2-K92, and pTH202 and the T and Vir regions.

TABLE 2. Mutants containing Tn*5* or cartridge insertions in pTiC58

Mutant[a]	Insertion or deletion site coordinate (kb)[b]	Virulence[c] on:		
		Tomato	Sunflower	Kalanchoë
JK503	2.6	−	−	−
JK504	7.0	−	−	−
JK516	11.9	+	+	+
JK517	11.9	+	+	+
JK518	12.7	(+)	−	−
JK519	12.7	(+)	−	−
JK505	13.4	(+)	(+)	−
JK521	14.1	(+)	(+)	−
JK506	14.3	(+)	(+)	(+)
JK507	15.3	+	+	+
JK520	16.2	+	+	+
JK522	16.2–19.4	+	+	+
JK515	17.0	+	+	+
JK508	19.6	+	+	+
JK509	20.4	+	+	+
JK510	20.6	+	+	+
JK511	20.8	+	+	+
JK512	22.1	+	+	+

[a] All mutants of pTiC58 Tra[c] were harbored in LBA4301. Mutants JK516, JK517, JK518, JK519, and JK520 were the result of CTN cartridge emplacement. JK517 and JK519 contained CTN insertions in orientations opposite to those in JK516 and JK518. JK515 is an *nptII* cartridge insertion. JK522 is a 3.2-kb deletion and CTN cartridge insertion mutant. All others are Tn*5* insertion mutants.

[b] Distance from the left border of *Kpn*I fragment 11.

[c] Symbols: +, tumors similar to wild type; (+), small slow-growing tumors at a few inoculation sites; −, no visible tumors.

that the affected gene(s) belongs to the same *trans*-acting complementation group. This locus aligns with the *virE* locus of the octopine plasmid pTiAch5 on the basis of DNA homology, nucleotide sequence, and complementation of this region (9; T. Hirooka, P. Rogowsky, and C. Kado, submitted for publication), and therefore may be the pTiC58 counterpart of the pTiAch5 *virE* gene (16). In the interest of uniformity for Vir region genetic nomenclature, we have designated the above complementation group *virE*, although further evidence will be needed to establish its true genetic identity.

Protein encoded by *virE*. To identify proteins encoded by *virE*, analyses were carried out in *E. coli* minicells. *Kpn*I fragment 9 (Fig. 4) cloned in pUCD9p produced polypeptides of 8 (not shown) and 69 kDa (Fig. 6, lane j). When *Kpn*I fragment 9 containing either JK516 or JK517 was analyzed, the 69-kDa protein was still produced (not shown). Proteins were not observed that were encoded by *Kpn*I fragment 9 containing pJK518 (Fig. 6, lane a) or pJK519 (Fig. 6, lane b) in pUCD2 with CTN cartridges inserted in the left end of *virE*; however, based on promoter activity in *Bam*HI fragment 32, proteins of less than 10 kDa would be expected. *Kpn*I fragment 9 containing pJK505 (Fig. 6, lane e) or pJK521 (Fig. 6, lane f) in pSa4 produced truncated proteins

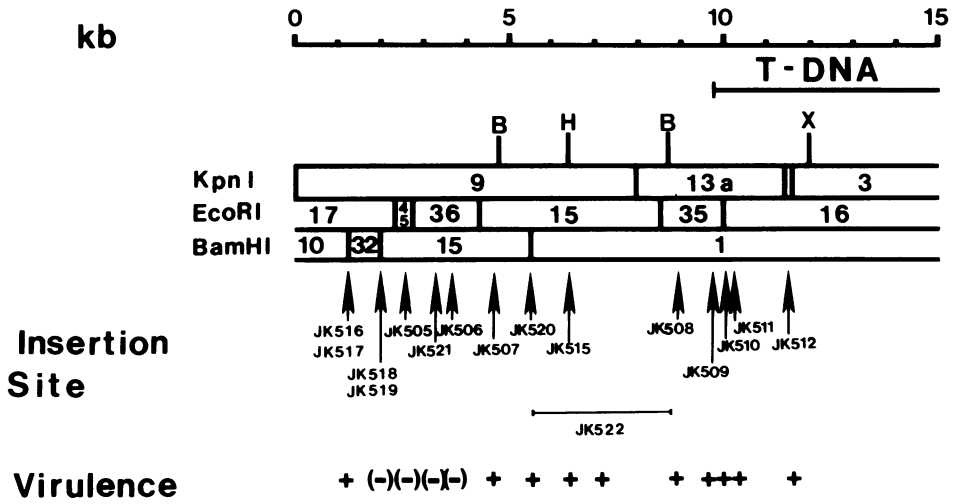

FIG. 4. Restriction map of the right-hand section of the Vir region and the left side of the T-DNA showing the sites of Tn*5* and cartridge insertion and a deletion (JK522) filled by the insertion of a CTN cartridge. Virulence phenotypes: +, virulent; (−), attenuated or avirulent, depending on host plant. Abbreviations: H, *Hin*dIII; X, *Xba*I; B, *Bgl*II.

TABLE 3. Complementation analysis of Tn*5* and CTN insertion mutants for virulence on different host plants

Mutant[a]	Complementing plasmid[b]	Virulence[c] on:		
		Tomato	Sunflower	Kalanchoë
JK518	None	(+)	−	−
JK518	pUCD2-K9$_1$	+	+	+
JK518	pUCD2-K9$_2$	+	+	+
JK518	pTH303	(+)	−	−
JK518	pTH60-57	(+)	(+)	−
JK518	pTH60-59	(+)	(+)	(+)
JK505	None	(+)	(+)	−
JK505	pUCD2-K9$_1$	+	+	+
JK505	pUCD2-K9$_2$	+	+	+
JK505	pTH303	(+)	(+)	−
JK505	pTH60-57	(+)	(+)	−
JK505	pTH60-59	(+)	(+)	(+)
JK506	None	(+)	(+)	(+)
JK506	pUCD2-K9$_1$	+	+	+
JK506	pUCD2-K9$_2$	+	+	+
JK506	pTH303	(+)	(+)	(+)
JK506	pTH60-57	(+)	(+)	(+)
JK506	pTH60-59	(+)	(+)	(+)

[a] All mutants of pTiC58 Tra[c] were harbored in LBA4301.

[b] Cloned fragments of pTiC58 in complementing plasmids are shown in Fig. 5.

[c] Virulence phenotypes of merodiploids on indicated host plants. Symbols: +, wild-type tumors; (+), small slow-growing tumors at a few inoculation sites (23); −, no visible tumors.

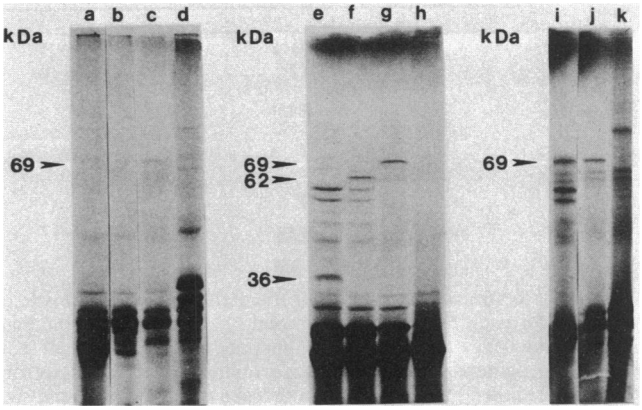

FIG. 6. Analysis of proteins in *E. coli* minicells. Proteins were separated by electrophoresis in 10% polyacrylamide–2% sodium dodecyl sulfate gels (22). Lane a, pTH303, which is pUCD2 containing subcloned *Kpn*I-9 with the CTN cartridge from pJK518; lane b, same as a, but the CTN cartridge is in the opposite orientation (pJK519 subclone); lane c, pUCD2-K9$_2$; lane d, pUCD2; lane e, pSa4 containing subcloned *Kpn*I-9 with Tn*5* from pJK505; lane f, pSa4 containing subcloned *Kpn*I-9 with Tn*5* from pJK521; lane g, pA16 (Table 1), pSa4 containing *Kpn*I-9; lane h, pSa4; lane i, pUCD9p containing subcloned *Kpn*I-9 with Tn*5* from pJK506; lane j, pUCD9p-K9$_2$; lane k, pUCD9p.

of 36 and 62 kDa, respectively. pJK506 in pUCD9p (Fig. 6, lane i) produced a protein indistinguishable in size from that of the wild-type 69-kDa *virE* protein. Nucleotide sequence analysis indicated that the Tn*5* insertion of JK506 had inserted five codons from the carboxyl terminus of this protein (Hirooka et al., submitted). Since JK506 displayed altered host specificity, the expected slightly smaller protein resulting from the Tn*5* insertion near the carboxyl terminus of the 69-kDa protein may still function but less effectively than the wild-type 69-kDa counterpart.

Virulence phenotype of mutations in *virE*. Mutants carrying mutations in the *virE* locus were characterized for their ability to induce tumors on tomato, potato, and jimson weed (*Solanaceae* family); sunflower, lettuce, and bachelor button (*Compositae* family); kalanchoë (*Crassulaceae* family); and *Vinca minor* (*Apocynaceae* family) (Fig. 7, Table 4). In comparison with wild-type pTiC58, which typically caused large tumors on all the test plants, mutant JK518 was a virulent on the three host plants of the *Compositae* family and on kalanchoë and *V. minor*. However, JK518 remained virulent on the three *Solanaceae* hosts, inducing tumors that were smaller and less frequent than those of the wild type. Mutants JK505 and JK521, which produced 36- and 62-kDa truncated proteins, respectively, were avirulent on kalanchoë and *V. minor*, but remained essentially virulent, inducing smaller tumors than wild-type pTiC58 on *Compositae* plants, except that JK505 was avirulent on lettuce. Mutant JK506, which produced the 69-kDa truncated protein, was virulent on all host plants tested (except *V. minor*), producing smaller tumors than the wild type. The frequency of tumor formation on kalanchoë was much reduced compared with that on other host plants inoculated with JK506. Interestingly, the frequency of tumor formation at inoculation sites on host plants in the *Solanaceae* and the *Compositae* families increased as the size of the truncated proteins increased toward the size of the wild-type 69-kDa protein.

DISCUSSION

The Vir region defined by transposon mutagenesis occupies a sector of the pTiC58 plasmid of approximately 30 kb. Here, we have defined the right boundary of the Vir region to be 6 kb counterclockwise from the left border of the T region (Fig. 3). Therefore, the right boundary is not contiguous with the T region. We have identified a gene in the *virE* operon that defines the right boundary and encodes a protein of 69

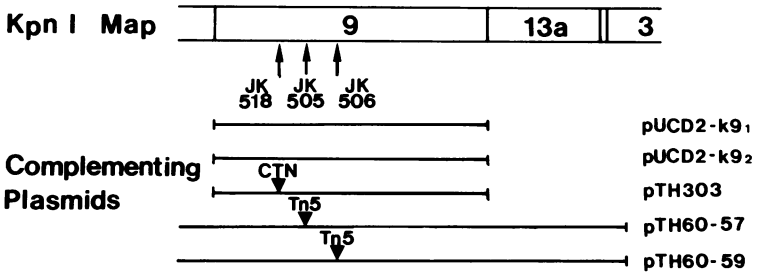

FIG. 5. *Kpn*I map showing CTN and Tn*5* insertion sites of mutants JK518, JK505, and JK506. The bars below the map indicate the fragments which are contained in complementing plasmids. The CTN arrowhead represents insertion of the CTN cartridge; the Tn*5* arrowheads represent insertion of Tn*5*. The designations given to each are indicated to the right.

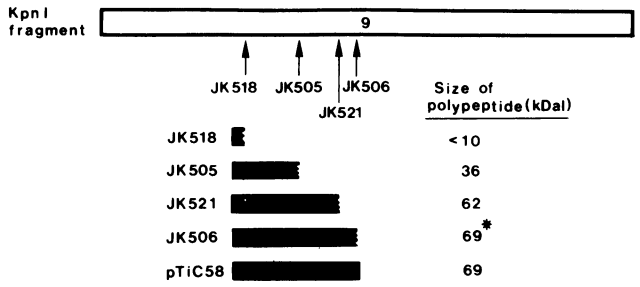

FIG. 7. Restriction map of *Kpn*I fragment 9 showing the site of mutation for each JK mutant. The solid black bars below the map indicate the sizes of the protein encoded by each mutant and wild-type fragment. The sizes of the proteins were calculated from the results shown in Fig. 6. Protein 69* is essentially the same size when the Tn*5* insertion is included.

kDa. Modification of this protein by different degrees of truncation clearly affects the host range of *A. tumefaciens* and the frequency of tumor formation. The resolution of the insertional mutations and deletion in this study is in the order of about 700 bp. There are some "gaps" between certain insertions, such as between JK506 and JK507, that are slightly larger than 700 bp and might permit room for a yet undefined gene. A promoter for the gene encoding this protein has been located in *Bam*HI fragment 32 (Fig. 8), and nucleotide sequence analysis of this boundary region indicates that the 3′ end of *virE* is about 300 bp left of the Tn*5* insertion site of JK507 (Fig. 4) (Hirooka et al., submitted).

We have assigned the designation *virE* to the operon next to the right boundary of the pTiC58 Vir region. This is consistent with the assignment of *virE* in the octopine plasmids pTiAch5 (16) and pTiA6NC (17, 22). The virulence of a *virE* mutant of pTiAch5 (16) and pTiA6NC (22) was abolished or attenuated on tomato, sunflower, tobacco, and kalanchoë, somewhat like the *virE* mutants of pTiC58 in our case. Like octopine *virE* mutants, mutants JK518, JK519, JK521, and JK505 of pTiC58 had attenuated virulence on tomato and were avirulent or weakly virulent on sunflower, depending on the site of the insertional mutation. Comparative DNA sequence analysis showed that the *virE* of pTiC58 has strong homology to the *virE* of pTiA6NC (Hirooka et al., submitted; S. Winans and E. Nester, manuscript in preparation). This suggests that the *virE* operon is conserved among pTi plasmids. The genetic complementation between the *virE* loci of octopine and nopaline pTi plasmids (Hirooka et al., submitted) seems to support this notion. Mutations JK516 and JK517 did not affect virulence.

TABLE 4. Virulence phonotype of mutants on various host plants

Host plant	Tumor size[a] and frequency (%)[b]			
	JK518	JK505	JK521	JK506
Tomato	+ (25)	+ (42)	+ (55)	+ (68)
Potato	+ (25)	+ (75)	+ (60)	+ (75)
Jimson weed	+ (75)	+ (75)	+ (75)	+ (100)
Sunflower	− (0)	+ (9)	+ (31)	+ (65)
Lettuce	− (0)	− (0)	+ (25)	+ (50)
Bachelor button	− (0)	+ (16)	+ (20)	+ (33)
Kalanchoë	− (0)	− (0)	− (0)	+ (2)
V. minor	− (0)	− (0)	− (0)	− (0)

[a] Symbols: +, small, slow-growing tumors as defined previously (24); −, no visible tumors. None of the mutants formed wild-type (pTiC58) tumors.

[b] Calculated as (sites with tumors/sites inoculated) × 100. The frequency with pTiC58 was 100% for all host plants tested.

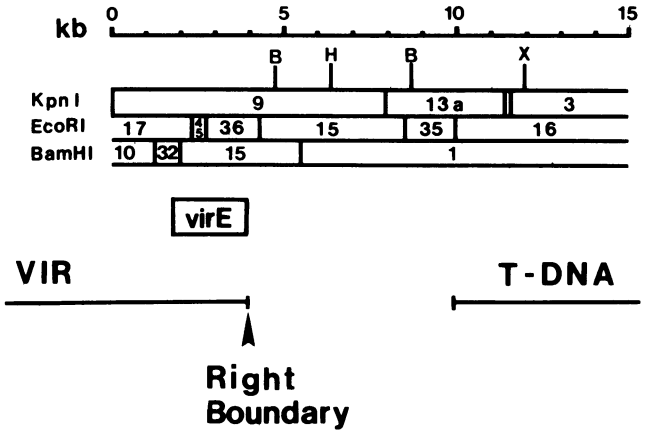

FIG. 8. Restriction map of a portion of pTiC58 showing the right boundary of the Vir region and the location of the *virE* gene. Abbreviations: B, *Bgl*II; H, *Hin*dIII; X, *Xba*I.

We showed that *virE* encodes a 69-kDa protein in *E. coli* minicells (Fig. 6), which is transcribed from left to right; the direction of transcription is indicated by the protein analysis, in which the size of this protein was decreased by truncation relative to the site of mutation from right to left in *virE*. The estimated size of the *virE* operon is about 2 kb, and it extends from *Bam*HI fragment 32 just to the middle of *Eco*RI fragment 36 (Fig. 8), based on the results of complementation tests and protein size. As stressed above, the *virE* protein is required for complete virulence expression of *Agrobacterium* species, and mutations truncating the *virE* protein result in narrowing the host range of *A. tumefaciens* and lowering the frequency of infection, depending on the size of the protein. It is therefore interesting that this protein might have several domains either mono- or multimerically that function in increasing the efficiency of the infection process in some hosts (*Compositae* family, kalanchoë, and *V. minor*) but not in others (*Solanaceae* family). Clearly, the protein encoded by *virE* is required for maintaining virulence efficiency.

Recently, Gardner and Knauf (10) claimed that the *virE* locus of pTiA6NC and pTiB6806 may be directly involved in the integration of the T-DNA or foreign DNA flanked by T-DNA borders into the host plant genome. However, we showed here that the *virE* gene encoding the 69-kDa protein, when truncated extensively, can still function in the formation of tumors in the more sensitive group of host plants (*Solanaceae*). Thus, this *virE* product may not be directly involved in T-DNA integration, but rather in some aspect of infection efficiency.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant CA-11526 from the National Cancer Institute and by a grant from the Competitive Research Grants Office, Science and Education Administration, U.S. Department of Agriculture.

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