JOURNAL OF BACTERIOLOGY, May 1981, p. 484-493
0021-9193/81/050484-10$02.00/0

Vol. 146, No. 2

Relationship Between the Limited and Wide Host Range Octopine-Type Ti Plasmids of *Agrobacterium tumefaciens*

MICHAEL F. THOMASHOW,† VIC C. KNAUF, AND EUGENE W. NESTER*

Department of Microbiology and Immunology, University of Washington, Seattle, Washington 98195

Received 17 October 1980/Accepted 27 February 1981

The relationship between the limited host range octopine Ti plasmids and the wide host range octopine Ti plasmids pTiB$_6$806 and pTiA6 was studied. The limited host range Ti plasmids shared extensive deoxyribonucleic acid homology; pTiAg63 and pTiAg162 were essentially completely homologous with pTiAg158 while pTiAg57 shared approximately 64% homology with pTiAg158. In contrast, the limited host range octopine Ti plasmids only shared 6 to 15% homology with the wide host range octopine Ti plasmid pTiB$_6$806. Thus, limited and wide host range octopine Ti plasmids comprise distinct families of plasmids. The deoxyribonucleic acid homology shared between the limited host range Ti plasmids and pTiB$_6$806, however, was distributed over some 50% of pTiB$_6$806, suggesting that both families of plasmids evolved from a common progenitor plasmid. The limited host range Ti plasmids showed relatively strong homology with pTiB$_6$806 *Hpa*I fragment 7, a region which codes for octopine utilization by the bacterium, but showed only weak homology with pTiB$_6$806 *Hpa*I fragment 12, a region required for virulence. In addition, homology between the limited host range octopine Ti plasmids and the "common deoxyribonucleic acid," sequences shown to have a central role in plant cell transformation, was barely detectable when stringent hybridization conditions were used. We therefore conclude that a highly conserved version of the common deoxyribonucleic acid is not required for crown gall tumorigenesis on all plant species.

The Ti plasmids of *Agrobacterium tumefaciens* are a diverse group of large plasmids responsible for causing crown gall, a neoplastic disease of dicotyledonous plants. Plant cell transformation is known to involve the stable transfer of a portion of the Ti plasmid, the T-DNA, from bacterium to plant (6, 43). In two tumor lines studied, the T-DNA has been shown to be covalently linked to plant nuclear DNA (8, 41, 46, 48); other studies suggest that the integration of T-DNA into the plant genome is a common feature of crown gall (40, 43). In all tumor lines examined, the T-DNA is composed in part of Ti plasmid sequences termed the "common DNA" (40, 43, 47). The common DNA sequences have been found in all previously described Ti plasmids (5, 12, 19) and are believed to encode the "oncogenes" active in the transformation process (5, 12, 15, 34, 40).

In addition to oncogenicity, the Ti plasmids code for a number of functions (14, 20, 25, 43, 44), including the determination of host range (28, 42) and bacterial utilization and tumor production of unusual guanido amino acids, primarily octopine, nopaline, and agropine (2, 16, 18, 32). DNA homology studies have suggested that the Ti plasmids that code for octopine utilization are a highly conserved group of plasmids (9); the *Sma*I, *Bst*I, *Eco*RI and *Hin*dIII restriction endonuclease digestion patterns of these plasmids are virtually identical (36, 40; unpublished data). Recently, however, we described a group of octopine-type Ti plasmids that were atypical in two respects (42). First, the *Bst*I restriction patterns of these Ti plasmids were nearly identical but bore little resemblance to the previously described octopine Ti plasmids. Second, the unusual octopine Ti plasmids coded for a limited host range; i.e., strains of *Agrobacterium* harboring these Ti plasmids could only form tumors on a limited number of plants tested (42). In contrast, the typical octopine Ti plasmids code for a wide host range. In this report, we further describe similarities and differences between the limited and wide host range octopine Ti plasmids.

MATERIALS AND METHODS

Bacterial strains. The strains of *Agrobacterium* sp. used in this study and the Ti plasmids they harbor are listed in Table 1. All strains were maintained on nutrient agar plates (Difco).

† Present address: Department of Bacteriology and Public Health, Washington State University, Pullman, WA 99164.

TABLE 1. *Strains of Agrobacterium sp.*

Strain	Plasmid(s)	Relevant phenotype[a]	Derivation
A208	pAtC58; pTiT37	WHR, NOC	Sciaky et al. (36)
A277	pAtC58; pTiB$_6$806	WHR, OCC	Sciaky et al. (36)
Ag57Tr	pAtC58; pTiAg57	LHR, OCC	Thomashow et al. (42)
Ag63Tr	pAtC58; pTiAg63	LHR, OCC	Thomashow et al. (42)
Ag158Tr	pAtC58; pTiAg158	LHR, OCC	Thomashow et al. (42)
Ag162Tr	pAtC58; pTiAg162	LHR, OCC	Thomashow et al. (42)
Ag86Tr	pAtC58; pTiAg86	WHR, OCC	This study
Ag2/6Tr	pAtC58; pTiAg2/6	WHR, NOC	This study
Ag2/6	pTiAg2/6; other cryptic plasmids	WHR, NOC	Wild-type *Agrobacterium* sp. from C. G. Panagopoulos
Ag86	pTiAg86; other cryptic plasmids	WHR, OCC	Wild-type *Agrobacterium* sp. from C. G. Panagopoulos
A136	pAtC58	Avirulent	Sciaky et al. (36)

[a] WHR, Wide host range (42); LHR, limited host range (42); NOC, nopaline catabolism; OCC, octopine catabolism.

Isolation of Ag2/6Tr and Ag86Tr. Total plasmid DNA was isolated from Ag2/6 (a nopaline-catabolizing strain) and Ag86 (an octopine-catabolizing strain) as described below and was used to transform *A. tumefaciens* A136 by standard procedures (21). Nopaline- or octopine-utilizing transformants were isolated by selection on AB minimal agar (4), with nopaline or octopine (400 μg/ml) serving as the sole carbon and nitrogen source. The parental strains Ag2/6 and Ag86 and their respective transformants Ag2/6Tr and Ag86Tr all have a wide range (V. Knauf and C. Panagopoulos, unpublished data).

Plasmid isolation. Strains of *Agrobacterium* sp. were grown overnight in either mannitol-glutamate broth (35) or a 1:1 mixture of mannitol-glutamate–L broth (31). Plasmid was then isolated by the Currier-Nester procedure (10) without the addition of magnesium phosphate. The isolation of the recombinant molecules pNW31C-2,19-1 and pNW31C-8,29-1 from the *Escherichia coli* strains in which they were harbored was as previously described (40); since the initial description of the isolation of these recombinant molecules, they have been transferred from *Escherichia coli* χ1776 to *E. coli* HB101.

In vitro labeling of DNA. DNA was labeled with ^{32}P by the nick-translation reaction (29) as modified (40).

DNA solution hybridization experiments. Solution hybridizations were performed by the method of Currier and Nester (9). Plasmid DNA was sheared in a French pressure cell at 12,000 lb/in^2 and dissolved (0.6 μg) in 20 μl of 0.15 M phosphate buffer containing ^{32}P-labeled probe DNA (18 to 20 pg). The samples were sealed in capillary pipettes, boiled for 5 min, and incubated at 68°C for 12 or 24 h. The percentage of probe DNA that had reassociated was assayed by hydroxyapatite chromatography; probe reassociation in the presence of heterologous DNA (salmon sperm DNA) was only 1 to 4%.

Restriction endonuclease digestion. The restriction endonucleases *Hpa*I (Bethesda Research Laboratories), *Bam*HI (Boehringer-Mannheim), and *Sma*I (Boehringer-Mannheim) were used as recommended by the suppliers. *Eco*RI was isolated as described (1) and used in a reaction buffer consisting of 10 mM Tris (pH 7.6), 6 mM $MgCl_2$, and 100 mM NaCl at 37°C.

Agarose gel electrophoresis and preparation of nitrocellulose filters. Restriction digests were fractionated by electrophoresis through 0.7% agarose horizontal slab gels (0.5 cm thick) in 80 mM Tris–40 mM sodium acetate–4 mM EDTA, pH 8.0 (adjusted with glacial acetic acid). The gels were stained with ethidium bromide (0.5 μg/ml), illuminated with an ultraviolet light box (Model C-61; Ultra Violet Products, Inc.) and photographed with a MP 4 Polaroid Land Camera equipped with orange and ultraviolet filters.

Preparation of nitrocellulose filters and hybridization conditions. Agarose gels were treated with 0.5 M NaOH–0.8 M NaCl for 1 h followed by 0.5 M Tris-hydrochloride (pH 7.0)–1.5 M NaCl for 2 h with gentle rocking at all times. The DNA was then transferred to nitrocellulose by the Southern procedure (38), except that 10× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) was used in place of 20× SSC for the transfer. After the transfer (generally 24 h) the filters were rinsed in 2× SSC, air dried, and baked either at 68°C overnight or at 80°C for 2 h in a vacuum oven.

The baked filters were wet in 2× SSC and treated with a prehybridization solution consisting of 6× SSC and 10× Denhardt's solution (1× Denhardt's solution is 0.02% bovine serum albumin, 0.02% Ficoll, and 0.02% polyvinylpyrrolidone [11]) for 12 to 16 h at 68°C in polyethylene bags. This solution was then replaced with 5 to 10 ml of a hybridization solution composed of 6× SSC, 5× Denhardt's solution, 20 mM Tris-hydrochloride (pH 7.6), 0.1% sodium dodecyl sulfate, 20 μg of sheared denatured steelhead trout sperm DNA per ml, 2 mM EDTA, and ^{32}P-labeled probe DNA, unless noted otherwise. The filters were incubated for 4 h at 68°C before the addition of the denatured (100°C for 5 min) ^{32}P-labeled DNA probe. The filters were then incubated for an additional 24 to 36 h at 68°C. After hybridization, the filters were washed in 6× SSC–0.2% sodium dodecyl sulfate–5 mM EDTA for 2 h at 68°C with four changes of the solution,

unless noted otherwise. The criterion of this hybridization procedure is approximately T_m − 41°C. Usually the filters were washed for an additional 30 min in 0.3× SSC–0.1% sodium dodecyl sulfate at 68°C. In these cases, the criterion of the hybridization was much more stringent (approximately T_m − 17°C). The T_m values were calculated by the method of Howley et al. (23), assuming that the Ti plasmids are 59% G + C (37). After hybridization and washing, the filters were dried and autoradiographed at −70°C for 1 to 24 h with Kodak RP-X-Omat film and a Dupont Cronex Lightning-Plus 7 E intensifying screen.

RESULTS

Relationship between the limited and wide host range octopine Ti plasmids. DNA homology studies have shown that the wide host range octopine Ti plasmids pTiB$_6$806, pTiA6, pTi15955 and pTiB2A are at least 90% homologous (9). The limited host range octopine Ti plasmids also share extensive homology among themselves. pTiAg63 and pTiAg162 are essentially completely homologous with pTiAg158, whereas pTiAg57 shows some 64% homology with pTiAg158 (Table 2). The limited host range octopine Ti plasmids, however, only share some 6 to 15% homology with the wide host range octopine Ti plasmid pTiB$_6$806 and only 9 to 15% homology with pTiT37, a wide host range nopaline Ti plasmid.

We further examined the relationship between the limited and wide host range octopine Ti plasmids by using a second experimental approach. *Hpa*I and *Sma*I digests of pTiB$_6$806 were fractionated by agarose gel electrophoresis, and the fragments were transferred to nitrocellulose filters. The filters were then cut into strips and hybridized with ^{32}P-labeled pTiB$_6$806, pTiAg57, pTiAg162, and pTiT37. The results of these experiments (Fig. 1 and 2) indicate that the homology between the limited host range Ti plasmids and pTiB$_6$806 is distributed over a large portion, approximately 50%, of pTiB$_6$806. The intensity of hybridization between the limited host range Ti plasmids and the restriction fragments of pTiB$_6$806, however, was much weaker than that of the homologous reaction (i.e., using ^{32}P-pTiB$_6$806 as the probe). Even when the hybridization and washing procedures were con-

TABLE 2. *DNA homology between the limited and wide host range Ti plasmids*[a]

Plasmid	Homology (%) with:		
	pTiAg158	pTiB$_6$806	pTiT37
pTiAg57	64	6	13
pTiAg63	98	12	9
pTiAg158	100	14	15
pTiAg162	90	15	11
pTiB$_6$806		100	15
pTiT37		15	100

[a] The Ti plasmids pTiAg158, pTiB$_6$806, and pTiT37 were labeled in vitro to specific activities of 1.7×10^8, 6.5×10^7, and 1.0×10^8 cpm/μg, respectively. The percent homologies between the probes and the indicated plasmids were then determined as described in the text.

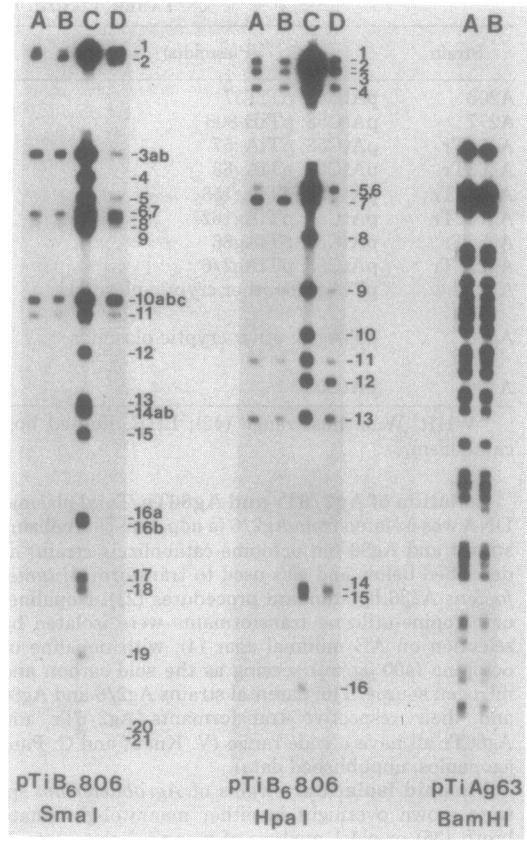

FIG. 1. *Hybridization of the limited host range Ti plasmids pTiAg57 and pTiAg162 with pTiB$_6$806 and pTiAg63. SmaI or HpaI digests of pTiB$_6$806 and BamHI digests of pTiAg63 were fractionated by agarose gel electrophoresis and transferred to nitrocellulose as described in the text; 1 μg of digested Ti plasmid was loaded into a 2.5-cm wide well. SmaI fragments 1 and 2 and HpaI fragments 1 through 4 were fractionated for a longer period of time before transfer to nitrocellulose than were the other SmaI and HpaI fragments. The filters were then cut into strips and hybridized with pTiAg162 (2.0×10^8 cpm/μg) (A), pTiAg57 (2.0×10^8 cpm/μg) (B), pTiB$_6$806 (2.0×10^8 cpm/μg) (C), or pTiT37 (1.9×10^8 cpm/μg) (D) at a probe concentration of 10 ng/ml. Conditions were as described in the text except that during hybridization and the 2 h of washing, the SSC concentration was 3× SSC. The filters were given a high-stringency wash in 0.3× SSC and autoradiographed.*

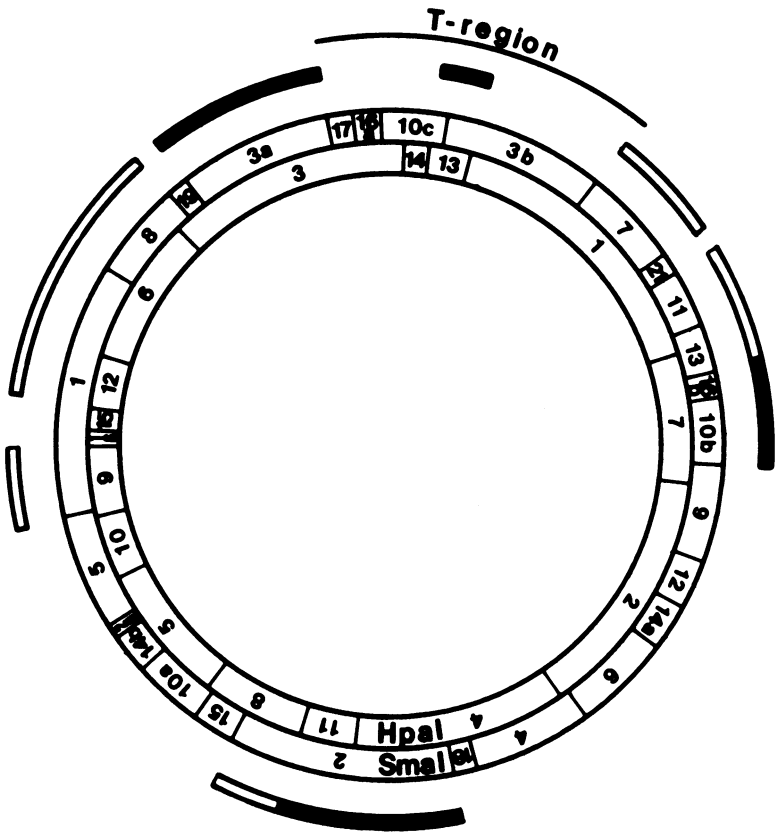

FIG. 2. *Regions of homology between the limited host range octopine Ti plasmids and the wide host range Ti plasmid pTiB$_6$806. The HpaI and SmaI restriction maps of pTiB$_6$806 have been published (7). All areas of pTiB$_6$806, except the T-region, showing relatively strong (■) or weak (□) hybridization with pTiAg57 and pTiAg162 were determined from the data presented in Fig. 1; homology with the T-region was determined from the data presented in Fig. 1, 3, 4, and 5. Areas of homology were based on stringent hybridization conditions. Very weak homology between the limited host range plasmids and the small HpaI and SmaI fragments of pTiB$_6$806 might not have been detected. In addition, the digests used in this study did not allow us to determine whether the limited host range Ti plasmids share homology with pTiB$_6$806 SmaI fragments 10a or 6.*

ducted under less stringent conditions (approximately $T_m - 41°C$, the intensity of hybridization did not change significantly; only *Sma*I fragment 8 and *Hpa*I fragments 5, 6, and 12 showed a slight increase in the intensity of hybridization (data not shown). The weak homology was due to peculiarities of the limited host range Ti plasmid probes since the probes hybridized with Southern transfers of *Bst*I digests of pTiAg63 as effectively as pTiB$_6$806 hybridized with itself (Fig. 1). These results suggest that only a fraction of a given pTiB$_6$806 restriction fragment showing homology with pTiAg57 or pTiAg162 is actually homologous with the probe. This conclusion is consistent with the results of the DNA solution hybridization experiments described above; i.e., only a small amount of homology was detected between the limited host range Ti plasmids and pTiB$_6$806.

Do the limited host range Ti plasmids have common DNA? All Ti plasmids studied have a highly conserved DNA element termed the common DNA (5, 12) which was originally defined as including pTiB$_6$806 *Sma*I fragments 16a, 17, and most of 10c (6; see Fig. 5). These sequences have a central role in plant cell transformation. They have been found in all tumor lines studied (40, 43, 46), and mutations introduced into this region of pTiB$_6$806 by insertion of Tn*5* have resulted in an altered tumor morphology (15). To establish whether the limited host range Ti plasmids contained the common DNA, we treated pTiAg57 and pTiAg162 with *Bam*HI, fractionated the digests by agarose gel

electrophoresis, and transferred the DNA fragments to nitrocellulose filters. Also included were *Bam*HI digests of pTiB$_6$806 and pTiT37, plasmids known to contain common DNA, and pTiAg86 and pTiAg2/6. These latter plasmids are wide host range octopine and nopaline type Ti plasmids, respectively, originally harbored by biotype 3 (24) strains of *Agrobacterium* spp. isolated from grapevines; the limited host range octopine plasmids were also isolated from biotype 3 strains of *Agrobacterium* spp. found in galls on grapevines. The filters with the bound DNA were then hybridized with ^{32}P-pNW31C-8,29-1, a recombinant plasmid containing essentially all of the common DNA sequences from pTiA6 inserted into pBR322. After hybridization, the filters were given a high-stringency wash (approximately T_m − 17°C) so that only well-matched sequences would remain annealed. As expected, pTiB$_6$806 *Bam*HI fragments 8 and 29 hybridized with the probe (Fig. 3). pTiT37 also showed extensive homology with the probe, as did pTiAg86 and pTiAg2/6. The hybridization was due to the common DNA sequences since the cloning vehicle, pBR322, did not hybridize with the plasmids (data not shown). In contrast, hybridization of the common DNA probe with the limited host range Ti plasmids was barely detectable. When the common DNA probe was hybridized with Southern transfers of *Sma*I-digested pTiAg57 or pTiAg162 (Fig. 4) or pTiAg63 and pTiAg158 (data not shown) under stringent hybridization conditions, again only faint hybridization was observed. We therefore conclude that the limited host range Ti plasmids do not contain a highly conserved version of the common DNA.

To determine whether the limited host range Ti plasmids contained sequences related to the common DNA, we hybridized the Southern transfers of the *Sma*I-digested Ti plasmids with pNW31C-8,29-1 under conditions of low stringency (T_m − 42°C). These conditions should allow DNA strands that are mismatched by approximately 30% to anneal. The autoradiograms show (Fig. 4) that there was an increase in hybridization of the common DNA probe with the limited host range plasmids, indicating that they have sequences related to the common DNA. To determine whether the limited host range Ti plasmids have DNA sequences related to the entire common DNA region or to only a portion of it, we fractionated a *Sma*I digest of pNW31C-8,29-1 by agarose gel electrophoresis and transferred the fragments to nitrocellulose. The filters were then cut into strips and hybridized with pTiAg57, pTiAg162, and pTiB$_6$806 at low and high stringencies. At high stringency (T_m − 17°C), hybridization of the limited host range plasmids with the filters was undetectable (Fig. 5), whereas at low stringency (T_m − 42°C), hybridization was observed with each of the *Sma*I fragments of pNW31C-8,29-1. We therefore conclude that the common DNA sequences that are related to the limited host range Ti plasmids are distributed over most of the common DNA region.

Homology between the limited host range Ti plasmids and the right half of the wide host range Ti plasmid T-region. The experiments described above show that the limited host range Ti plasmids have sequences homologous with pTiB$_6$806 *Hpa*I fragment 13, even under conditions of high stringency (Fig. 1). From the data presented, however, we could not conclude whether the limited host range Ti plasmids shared homology with the region of pTiB$_6$806 between *Hpa*I fragment 13 and *Sma*I fragment 7 (Fig. 5). We therefore digested the recombinant plasmid pNW31C-2,19-1 with *Eco*RI, separated the fragments by agarose gel electrophoresis, and transferred the DNA to nitrocellulose filters. The filters were then hybridized with pTiB$_6$806, pTiAg57, and pTiAg162 at low stringency (T_m − 42°C). The data show (Fig. 5) that there is essentially no homology between the limited host range Ti plasmid and the right end of the T-region of pTiB$_6$806.

Regions of homology between the limited host range Ti plasmids and pTiB$_6$806. In addition to the common DNA, other regions of pTiB$_6$806 were also expected to be homologous with the limited host range octopine plasmids. Both Ti plasmid types code for octopine catabolism by the bacterium (2, 32, 42). The region of pTiB$_6$806 which encodes the octopine catabolism genes is that of *Hpa*I fragment 7 (15, 25). Indeed, one of the strongest regions of homology between the limited host range Ti plasmids and pTiB$_6$806 is pTiB$_6$806 *Hpa*I fragment 7 (Fig. 1). pTiT37 does not code for octopine utilization and shows only slight homology with this region of pTiB$_6$806. Another region of homology that might be expected is *Hpa*I fragment 12. These sequences code for an as yet unknown virulence function; mutations in this region of pTiB$_6$806 result in avirulence (15, 34). Relatively strong homology with this region of pTiB$_6$806 has been found with nopaline Ti plasmids (13; see pTiT37 results [Fig. 1]) and the distantly related "hairy root" virulence plasmid of *Agrobacterium rhizogenes* (45). Surprisingly, homology between the limited host range Ti plasmids and this region of pTiB$_6$806 was only barely detectable (Fig. 1). Finally, a striking feature of the results presented in Fig. 1 is the similarity in hybridization patterns of pTiB$_6$806 with the limited host range Ti plasmids and pTiT37. These data

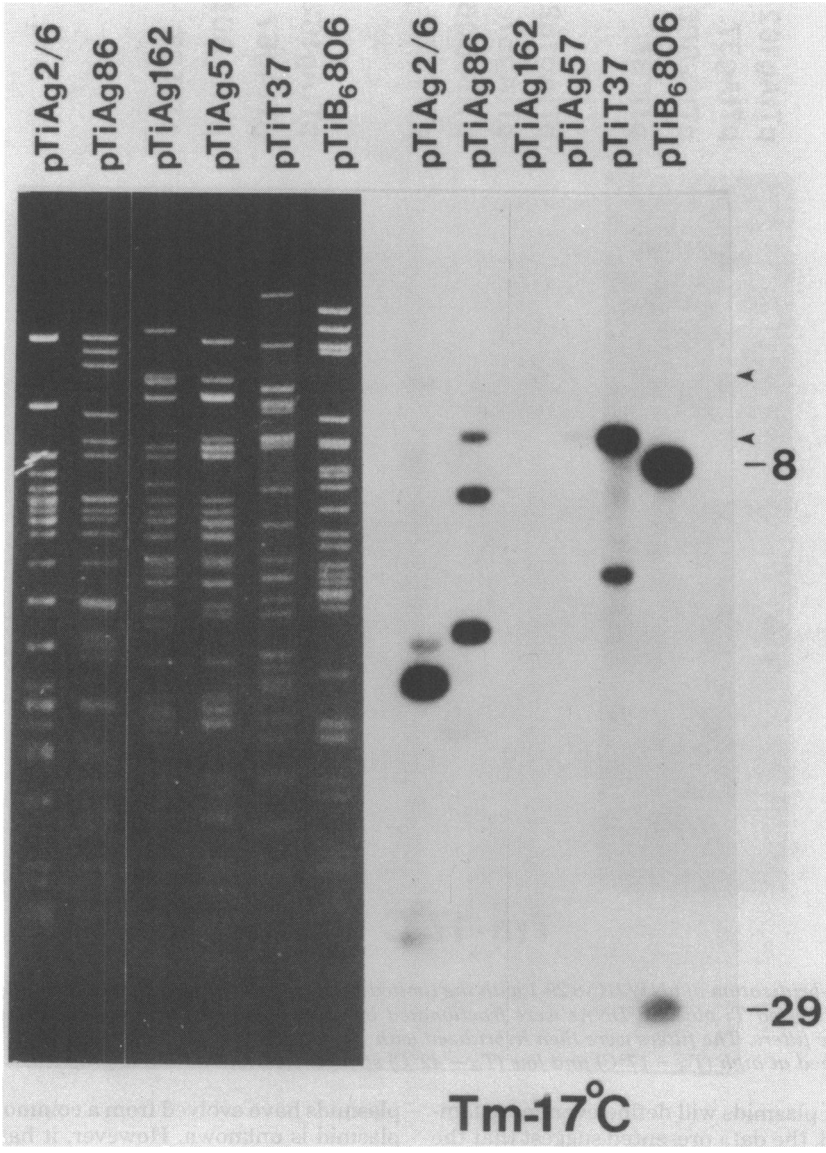

FIG. 3. *Hybridization of pNW31C-8,29-1 with the limited host range Ti plasmids and representative wide host range Ti plasmids. BamHI digested Ti plasmids were fractionated by agarose gel electrophoresis and transferred to nitrocellulose. The filters were then hybridized with the common DNA probe, ^{32}P-labeled pNW31C-8,29-1 (7×10^7 cpm/μg; 10 ng/ml), washed at high stringency (T_m − 17°C), and autoradiographed. The top and bottom arrows point to the weak hybridization seen with the digests of pTiAg162 and pTiAg57, respectively.*

suggest the possibility that a large portion of the sequences conserved in the limited and wide host range octopine Ti plasmids is also found in the nopaline Ti plasmids.

DISCUSSION

From the data presented in this report we conclude that two widely held beliefs about Ti plasmids must be modified. First, it has been suggested that the octopine Ti plasmids are a highly conserved group of plasmids (36). This notion, however, was based on the examination of relatively few Ti plasmid isolates. It is clear from the data presented here that the octopine Ti plasmids that code for a limited host range (42) are a distinct group of Ti plasmids distantly related to the previously described octopine Ti plasmids. We suspect that a survey of additional

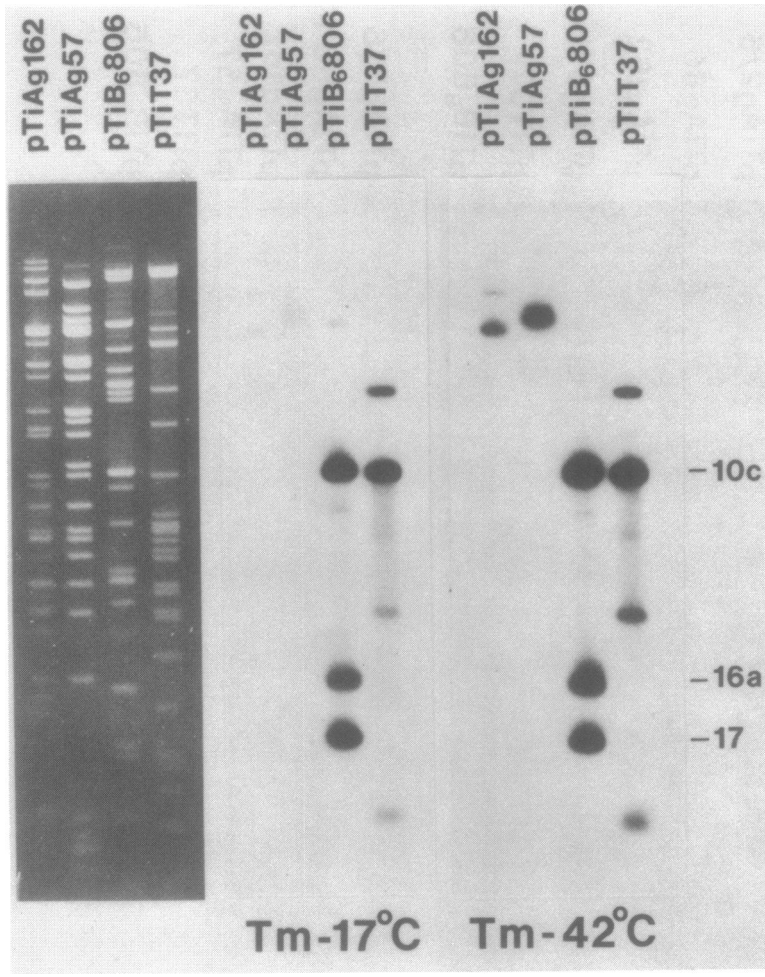

FIG. 4. *Hybridization of pNW31C-8,29-1 with the limited host range Ti plasmids at high and low stringencies. SmaI digested Ti plasmid DNAs were fractionated by agarose gel electrophoresis and transferred to nitrocellulose filters. The filters were then hybridized with ^{32}P-labeled pNW31C-8,29-1 (2.8×10^7 cpm/μg; 10 ng/ml), washed at high (T_m − 17°C) and low (T_m − 42°C) stringencies, and autoradiographed.*

octopine Ti plasmids will define even more families. Indeed, the data presented suggest that the wide host range octopine Ti plasmid pTiAg86 is distinct from both the limited Ti plasmids and the "common" wide host range octopine Ti plasmids; the *Bam*HI restriction pattern of pTiAg86 is different than that of both pTiB$_6$806 and the limited host range Ti plasmids (Fig. 3). Although the data indicate that the limited host range and common wide host range octopine Ti plasmids are distinct families of plasmids they do suggest that both plasmid types evolved from a common ancestral plasmid. This follows from the observation that the limited sequence homology shared between pTiB$_6$806 and the limited host range Ti plasmids is dispersed over a large portion of the plasmids. Whether or not all Ti plasmids have evolved from a common ancestral plasmid is unknown. However, it has been suggested that the nopaline Ti plasmids (13, 22), the virulence plasmid of *A. rhizogenes* (43), and pTiB$_6$806 have also evolved from a common progenitor plasmid.

A second widely held belief about Ti plasmids is that they all contain a highly conserved DNA element, the common DNA, which is responsible for tumor formation (5, 12, 15, 17, 34, 40). The tumors incited by the limited host range octopine Ti plasmids appear to be typical unorganized tumors; upon culturing, the tumor tissues are phytohormone independent and produce octopine (V. Knauf, unpublished data). Thus, it was expected that the limited host range Ti plasmids would contain the highly conserved

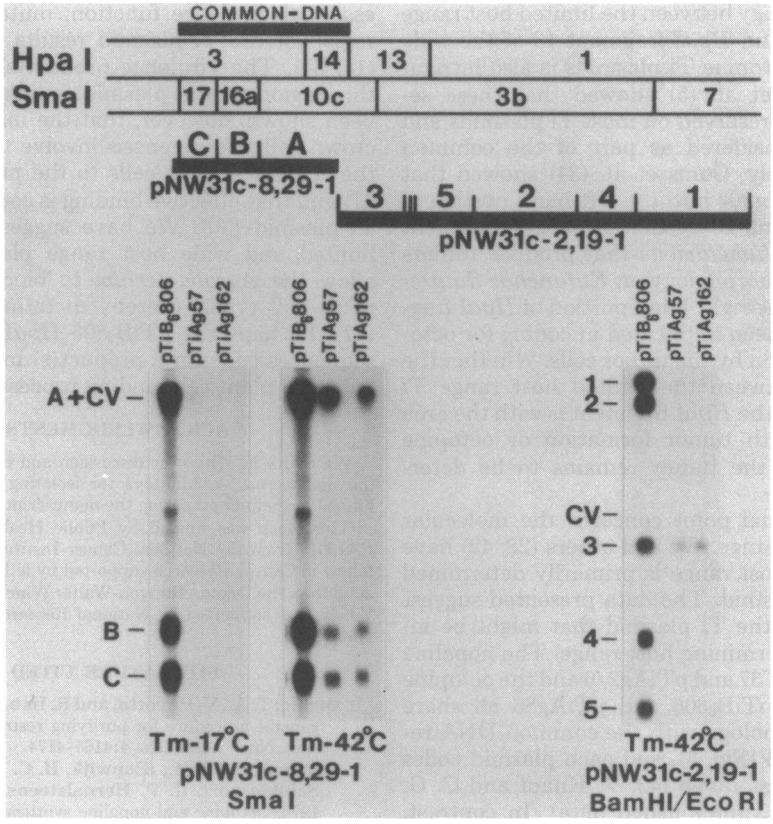

FIG. 5. *Homology between the limited host range Ti plasmids and the T-region of pTiA6. pNW31C-2,19-1 that had been digested with both BamHI and EcoRI and SmaI-digested pNW31C-8,29-1 were fractionated by agarose gel electrophoresis and transferred to nitrocellulose. The filters were then cut into strips and hybridized with in vitro-labeled pTiB$_6$806 (2.7×10^7 cpm/μg), pTiAg57 (3.2×10^7 cpm/μg), or pTiAg162 (5.0×17^7 cpm/μg); all probes were used at 5×10^5 cpm/ml. The filter strips were then washed at the indicated stringency and autoradiographed. Also shown are the HpaI and SmaI restriction maps of the T-region of pTiA6, the common DNA sequences, the Ti-plasmid sequences contained in the T-region clones pNW31C-8,29-1 and pNW31C-8,19-1, and the fragments generated by SmaI or BamHI/EcoRI digestion of the respective clones. Migration of the cloning vehicle (CV) pBR322 is indicated.*

common DNA. This was not the case. Hybridization between the limited host range octopine Ti plasmids and the common DNA was barely detectable when stringent hybridization conditions were used (Fig. 3 and 4). We therefore conclude that a highly conserved common DNA is not required for crown gall tumorigenesis on all plant species.

The full significance of this finding is not clear. The human papovavirus BK and simian virus 40 code for tumor antigens that are immunologically cross-reactive (39) and presumably have similar functions. Homology between these genes is not detected under standard conditions of hybridization (T_m − 25°C); the stringency must be lowered (T_m − 35°C) for DNA homology to be observed (3, 23, 33). Similarly, we detected homology between the limited host range Ti plasmids and the common DNA region of pTiB$_6$806 and pTiA6 when hybridization was carried out at low stringency (Fig. 4). If the limited host range Ti plasmid sequences that are related to the common DNA are transferred to the plant cells, it is possible that these sequences make a gene product(s) very similar to the transformation product(s) presumably encoded by the common DNA sequences. Alternatively, the oncogene(s) encoded by the limited host range octopine Ti plasmids might be different than those of the wide host range octopine plasmids; i.e., there might be more than one oncogene(s) that can induce the formation of unorganized crown gall tumors. A comparison of the T-DNA sequences and their expression in tumors incited by limited and wide host range octopine Ti plasmids should help clarify the situation.

The homology between the limited host range Ti plasmids and *HpaI* fragment 13 of the wide host range octopine Ti plasmids is also intriguing. Chilton et al. (5) showed that these sequences are preserved on most Ti plasmids and might be considered as part of the common DNA. Recently, Ooms et al. (34) showed that insertion of Tn*904* into the left-hand portion of *HpaI* fragment 13 results in weakly virulent strains of *A. tumefaciens* that produce tumors with altered morphology on *Kalanchoe daigremontiana*. The right-hand portion of *HpaI* fragment 13 has been implicated in coding for octopine production by the tumor cells. Whether the homology between the limited host range Ti plasmids and the *HpaI* fragment is with the area concerned with tumor formation or octopine synthesis by the tumor remains to be determined.

An additional point concerns the molecular basis of host range. We and others (28, 42) have shown that host range is primarily determined by the Ti plasmid. The data presented suggest two areas of the Ti plasmid that might be involved in determining host range. The nopaline plasmids pTiT37 and pTiAg2/6 and the octopine Ti plasmids pTiB$_6$806 and pTiAg86 all share extensive homology with the common DNA region of pTiA6 (Fig. 3), and each plasmid codes for a wide host range (42; V. Knauf and C. G. Panagopoulos, unpublished data). In contrast, the Ti plasmids pTiAg57, pTiAg63, pTiAg158, and pTiAg162 code for a limited host range (42) and have a more distantly related common DNA. The correlation of host range with the presence or absence of a highly conserved common DNA raises the possibility that the common DNA itself plays a role in determining host range. One could imagine that the gene product(s) active in transforming one plant species might not be active or as effective in transforming another plant species.

Another region of the Ti plasmid that might be involved in determining host range is the region of pTiB$_6$806 *HpaI* fragment 12. Essentially all of the Ti plasmids that have been examined have a high degree of homology with this region of pTiB$_6$806: these plasmids include the nopaline Ti plasmids pTiC58, pTiT37, pTi223 and pTiIIBV7 (13), the agropine Ti plasmid pTi542 (13) and the wide host range octopine plasmids (13; Fig. 1). The virulence plasmid of *A. rhizogenes* responsible for causing hairy-root disease also shows a high degree of homology with pTiB$_6$806 *HpaI* fragment 12 (45). The limited host range Ti plasmids, however, share little homology with this region of pTiB$_6$806 (Fig. 1). This observation is of interest since it has been shown that these sequences encode an essential virulence function; mutations in this region of the Ti plasmid results in avirulence (15, 34). The virulence product(s) encoded by this region of the plasmid is unknown. It has been shown, however, that the initial stages of crown gall tumorigenesis involve the binding of the *A. tumefaciens* cells to the plant cells (26, 27) and that effective binding is coded for by the Ti plasmid (30). We have suggested that the limited and wide host range plasmids might allow the *Agrobacterium* to bind to different plant cell types, thereby dictating host range (42). Perhaps the pTiB$_6$806 *HpaI* fragment 12 sequences code for a product(s) involved in the bacterial plant cell binding process.

ACKNOWLEDGMENTS

We thank F. White for discussion and critical reading of the manuscript, A. Montoya for isolating Ag86Tr, and R. Finical for help in preparing the manuscript.

This work was funded by Public Health Service grant CA13015 from the National Cancer Institute. M.F.T. was a fellow in Cancer Research supported by fellowship no. DRG-209F from the Damon Runyon-Walter Winchell Cancer Fund. V.C.K. was supported by National Research Service Award GM07270.

LITERATURE CITED

1. **Bickle, T. A., V. Pirrotta, and R. Imber.** 1977. A simple, general procedure for purifying restriction endonucleases. Nucl. Acids Res. **4**:4165–4174.
2. **Bomhoff, G., P. M., Klapwijk, H. C. M. Kester, R. A. Schilperoort, J. P. Hernalsteens, and J. Schell.** 1976. Octopine and nopaline synthesis and breakdown genetically controlled by a plasmid of *Agrobacterium tumefaciens*. Mol. Gen. Genet. **145**:177–181.
3. **Bond, S. B., P. M. Hawley, and K. K. Takemoto.** 1978. Characterization of BK virus and its comparison with polyoma virus. J. Virol. **28**:337–343.
4. **Chilton, M.-D., T. C. Currier, S. K. Farrand, A. J. Bendich, M. P. Gordon, and E. W. Nester.** 1974. *Agrobacterium tumefaciens* DNA and PS8 bacteriophage DNA not detected in crown gall tumors. Proc. Natl. Acad. Sci. U.S.A. **71**:3672–3676.
5. **Chilton, M.-D., M. H. Drummond, D. J. Merlo, and D. Sciaky.** 1978. Highly conserved DNA of Ti-plasmids overlaps T-DNA maintained in plant tumours. Nature (London) **275**:147–149.
6. **Chilton, M.-D., M. H. Drummond, D. J. Merlo, D. Sciaky, A. L. Montoya, M. P. Gordon, and E. W. Nester.** 1977. Stable incorporation of plasmid DNA into higher plant cells; the molecular basis of crown gall tumorigenesis. Cell **11**:263–271.
7. **Chilton, M.-D., A. L. Montoya, D. J. Merlo, M. H. Drummond, R. Nutter, M. P. Gordon, and E. W. Nester.** 1978. Restriction endonuclease mapping of a plasmid that confers oncogenicity upon *Agrobacterium tumefacien* strain B6-806. Plasmid **1**:254–269.
8. **Chilton, M.-D., R. K. Saiki, N. Yadav, M. P. Gordon, and F. Quetier.** 1980. T-DNA from *Agrobacterium* Ti-plasmid is in the nuclear DNA fraction of crown gall tumor cells. Proc. Natl. Acad. Sci. U.S.A. **77**:4060–4064.
9. **Currier, T. C., and E. W. Nester.** 1976. Evidence for diverse types of large plasmids in tumor-inducing strains of *Agrobacterium*. J. Bacteriol. **126**:157–165.
10. **Currier, T. C., and E. W. Nester.** 1976. Isolation of covalently closed circular DNA of high molecular weight from bacteria. Anal. Biochem. **66**:431–441.
11. **Denhardt, D. T.** 1966. A membrane-filter technique for

the detection of complementary DNA. Biochem. Biophys. Res. Commun. **23**:641–646.
12. **Depicker, A., M. Van Montagu, and J. Schell.** 1978. Homologous DNA sequences in different Ti-plasmids are essential for oncogenicity. Nature (London) **275**: 150–153.
13. **Drummond, M. H., and M.-D. Chilton.** 1978. Tumor-inducing (Ti) plasmids of *Agrobacterium* share extensive regions of DNA homology. J. Bacteriol. **136**:1178–1183.
14. **Engler, G., M. Holsters, M. Van Montagu, J. Schell, J. P. Hernalsteens, and R. Schilperoort.** 1975. Agrocin 84 sensitivity: a plasmid determined property in *Agrobacterium tumefaciens*. Mol. Gen. Genet. **138**: 345–349.
15. **Garfinkel, D. J., and E. W. Nester.** 1980. Mutants in *Agrobacterium tumefaciens* affected in crown gall tumorigenesis and octopine catabolism. J. Bacteriol. **144**:732–743.
16. **Goldman, A., D. W. Thomas, and G. Morel.** 1969. Sur le structure de la nopaline, metabolite anormale de certaines tissus de crown gall. C. R. Acad. Sci. (Paris) **268**:852–854.
17. **Gurley, W. B., J. D. Kemp, M. J. Albert, D. W. Sutton, and J. Collins.** 1979. Transcription of Ti plasmid derived sequences in three octopine-type crown gall tumor lines. Proc. Natl. Acad. Sci. U.S.A. **76**:2829–2832.
18. **Guyon, P., M.-D. Chilton, A. Petit, and J. Tempé.** 1980. Agropine in "null-type" crown gall tumors: evidence for generality of the opine concept. Proc. Natl. Acad. Sci. U.S.A. **77**:2693–2697.
19. **Hepburn, A., and J. Hindley.** 1979. Regions of DNA sequence homology between an octopine and a nopaline Ti-plasmid of *Agrobacterium tumefaciens*. Mol. Gen. Genet. **169**:163–172.
20. **Holsters, M., B. Silva, F. Van Vliet, C. Genetello, M. DeBlock, P. Dhaese, A. Depicker, D. Inze, G. Engler, R. Villarroel, M. Van Montagu, and J. Schell.** 1980. The functional organization of the nopaline *A. tumefaciens* plasmid pTiC58. Plasmid **3**:212–230.
21. **Holsters, M., D. de Waele, A. Depicker, E. Messens, M. Van Montagu, and J. Schell.** 1978. Transfection and transformation of *Agrobacterium tumefaciens*. Mol. Gen. Genet. **163**:181–187.
22. **Hooykass, P. J. J., H. Den Dulk-Ras, G. Ooms, and R. A. Schilperoort.** 1980. Interactions between octopine and nopaline plasmids in *Agrobacterium tumefaciens*. J. Bacteriol. **143**:1295–1306.
23. **Howley, P. M., M. A. Israel, M.-F. Law, and M. A. Martin.** 1979. A rapid method for detecting and mapping homology between heterologous DNAs. J. Biol. Chem. **254**:4876–4883.
24. **Kerr, A., and C. G. Panagopoulos.** 1977. Biotypes of *Agrobacterium radiobacter* var. *tumefaciens* and their biological control. Phytopath. Z. **90**:172–179.
25. **Koekman, B. P., G. Ooms, P. M. Klapwijk, and R. A. Schilperoort.** 1979. Genetic map of an octopine Ti-plasmid. Plasmid **2**:347–357.
26. **Lippincott, B. B., and J. A. Lippincott.** 1969. Bacterial attachment to a specific wound site as an essential stage in tumor initiation by *Agrobacterium tumefaciens*. J. Bacteriol. **94**:620–628.
27. **Lippincott, B. B., M. H. Whatley, and J. A. Lippincott.** 1977. Tumor induction by *Agrobacterium* involves attachment of the bacterium to a site on the plant cell wall. Plant Physiol. **59**:388–390.
28. **Loper, J. E., and C. I. Kado.** 1979. Host range conferred by the virulence-specifying plasmid of *Agrobacterium tumefaciens*. J. Bacteriol. **139**:591–596.
29. **Maniatis, T., A. Jeffrey, and D. G. Kleid.** 1975. Nucleotide sequence of the rightward operator of phage λ. Proc. Natl. Acad. Sci. U.S.A. **72**:1184–1188.
30. **Matthysse, A. G., P. M. Wyman, and K. V. Holmes.** 1978. Plasmid-dependent attachment of *Agrobacterium tumefaciens* to plant tissue culture cells. Infect. Immun. **13**:1080–1083.
31. **Miller, J. H.** 1972. Experiments in Molecular Genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
32. **Montoya, A. L., M.-D. Chilton, M. P. Gordon, D. Sciaky, and E. W. Nester.** 1974. Octopine and nopaline metabolism in *Agrobacterium tumefaciens* and crown gall tumor cells: role of plasmid genes. J. Bacteriol. **129**:101–107.
33. **Newell, N., C.-J. Lai, G. Khoury, and T. J. Kelly, Jr.** 1978. Electron microscope study of the base sequence homology between simian virus 40 and human papovavirus BK. J. Virol. **25**:193–201.
34. **Ooms, G., P. M. Klapwijk, J. A. Poulis, and R. A. Schilperoort.** 1980. Characterization of Tn*904* insertions in octopine Ti plasmid mutants of *Agrobacterium tumefaciens*. J. Bacteriol. **144**:82–91.
35. **Roberts, W. P., and A. Kerr.** 1974. Crown gall induction: serological reactions, isozyme patterns and sensitivity to mitomycin C and to bacteriocin of pathogenic and nonpathogenic strains of *Agrobacterium radiobacter*. Physiol. Plant Pathol. **4**:81–91.
36. **Sciaky, D., A. L. Montoya, and M.-D. Chilton.** 1977. Fingerprints of *Agrobacterium* Ti-plasmids. Plasmid **1**: 238–253.
37. **Sheikholeslam, S., B.-C. Lin, and C. I. Kado.** 1979. Multiple-size plasmids in *Agrobacterium radiobacter* and *A. tumefaciens*. Phytopathology **69**:54–58.
38. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
39. **Takemoto, K. K., and M. F. Mullarkey.** 1973. Human papovavirus, BK strain: biological studies including antigenic relationship to simian virus 40. J. Virol. **12**:625–631.
40. **Thomashow, M. F., R. Nutter, A. L. Montoya, M. P. Gordon, and E. W. Nester.** 1980. Integration and organization of Ti-plasmid sequences in crown gall tumors. Cell **19**:729–739.
41. **Thomashow, M. F., R. Nutter, K. Postle, M.-D. Chilton, F. R. Blattner, A. Powell, M. P. Gordon, and E. W. Nester.** 1980. Recombination between higher plant DNA and the Ti plasmid of *Agrobacterium tumefaciens*. Proc. Natl. Acad. Sci. U.S.A. **77**:6448–6452.
42. **Thomashow, M. F., C. G. Panagopoulos, M. P. Gordon, and E. W. Nester.** 1980. Host range of *Agrobacterium tumefaciens* is determined by the Ti-plasmid. Nature (London) **283**:794–796.
43. **Van Montagu, M., and J. Schell.** 1979. The plasmids of *Agrobacterium tumefaciens*, p. 71–95. *In* K. N. Timmis and A. Puhler (ed.), Plasmids of medical, environmental and commercial importance. Elsevier/North-Holland Biomedical Press, Amsterdam.
44. **Watson, B., T. C. Currier, M. P. Gordon, M.-D. Chilton, and E. W. Nester.** 1975. Plasmid required for virulence of *Agrobacterium tumefaciens*. J. Bacteriol. **123**:255–264.
45. **White, F. F., and E. W. Nester.** 1980. The relationship of the plasmids responsible for hairy root and crown gall tumorigenicity. J. Bacteriol. **144**:710–720.
46. **Yadav, N. S., K. Postle, R. K. Saiki, M. F. Thomashow, and M.-D. Chilton.** 1980. T-DNA of a crown gall teratoma is covalently joined to host plant DNA. Nature (London) **287**:458–461.
47. **Yang, F., A. L. Montoya, D. J. Merlo, M. H. Drummond, M.-D. Chilton, E. W. Nester, and M. P. Gordon.** 1980. Foreign DNA sequences in crown gall teratoma and their fate during loss of the tumorous traits. Mol. Gen. Genet. **177**:707–714.
48. **Zambryski, P., M. Holsters, K. Kruger, A. Depicker, J. Schell, M. Van Montagu and H. M. Goodman.** 1980. Tumor DNA structure in plant cells transformed by *A. tumefaciens*. Science **209**:1385–1391.