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T-DNA from *Agrobacterium* Ti plasmid is in the nuclear DNA fraction of crown gall tumor cells

(plant tumors/teratomas/fragmentation patterns/chloroplast DNA/mitochondrial DNA)

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ABSTRACT **The crown gall teratoma tumor line BT37, incited by *Agrobacterium tumefaciens* strain T37, has been found to contain part of the tumor-inducing plasmid, pTi T37, of the inciting strain. This foreign DNA segment, called T-DNA, is maintained at several copies per diploid tumor cell. We have examined subcellular DNA fractions from this tumor line in an effort to determine whether T-DNA is in chloroplasts, mitochondria, or nuclei. Tumor cell chloroplast DNA exhibited *Eco*RI and *Bst* I endonuclease cleavage patterns identical to those of normal tobacco chloroplast DNA. Tumor cell mitochondrial DNA exhibited a complex *Bst* I cleavage pattern that did not differ detectably from that of normal tobacco mitochondrial DNA. Southern blots of tumor chloroplast and mitochondrial cleavage products did not hybridize with labeled pTi T37 DNA, whereas blots of tumor cell nuclear DNA cleavage products hybridized strongly. We conclude that T-DNA is located not in chloroplasts or mitochondria but rather in the nuclear fraction of this tumor line.**

Crown gall tumors are produced by inoculation of plant wound sites with *Agrobacterium tumefaciens*. The oncogenic strains of this soil bacterium contain large plasmids (1), called Ti (tumor-inducing) plasmids, that carry genetic information essential for tumor induction (2, 3). Unlike normal plant tissue, axenic tumor tissue grows *in vitro* without exogenous auxin and cytokinin, and produces novel amino acid metabolites called opines that are specified by the Ti plasmid (4–6). These altered attributes are apparently ascribable to the presence of foreign DNA transmitted from the oncogenic bacterium to the plant cell. Transformed plant cells contain and maintain copies of a small part of the Ti plasmid called T-DNA (7–11). The exact boundaries of T-DNA on the Ti plasmid can vary (8, 11); thus it does not appear to resemble the bacterial transposons (12).

T-DNA is transcribed in tumor cells (13–15), and the transcripts have poly(A) (14) and are found on polysomes (16). Thus this foreign, presumably prokaryotic, genetic information is able to function in a eukaryotic cell.

The location of T-DNA in the crown gall tumor cell has been the subject of considerable speculation. Two arguments favor the chloroplast as a plausible site: the endosymbiont theory of the origin of chloroplasts (17) and the lack of 5-methylcytosine in chloroplast DNA (whereas nuclear DNA contains a substantial fraction) (17) seem to imply that T-DNA would find the chloroplast a less "foreign" environment than the plant nucleus. A hint that T-DNA might be located in the mitochondrial genome came from a study of the *Eco*RI endonuclease cleavage patterns of crown gall tumor mitochondrial DNA and normal plant mitochondrial DNA. The tumor mitochondrial DNA fingerprint lacked a fragment that was present in normal *Parthenocissus tricuspidata* mitochondrial DNA (18).

A final argument supporting the view that either chloroplast or mitochondrial DNA might harbor the T-DNA is the evidence that T-DNA can be lost from crown gall tumor cells. Braun and his collaborators (19, 20) showed that cloned crown gall teratoma tissue could, when grafted to the apex of a decapitated tobacco plant, give rise to normal-appearing shoots (19, 20), one of which was fertile and set seed. Tissues of the grafted shoot were found to retain tumorous potential, because they reverted to teratomatous growth when planted in tissue culture (19, 20) and the resulting tissue lines contained T-DNA (9). However, haploid anther-derived tissue from this shoot and selfed progeny plant tissue were found to possess characteristics of normal tobacco cells in vitro (19, 20) and to be free from T-DNA (9). The apparent loss of T-DNA at meiosis could be rationalized more readily if it were in plastids rather than joined to nuclear DNA.

The experiments described here were undertaken to test whether T-DNA is indeed located in chloroplast or mitochondrial DNA fractions. We have analyzed the same tumor line employed in Braun's grafting study (19, 20). The approach we used was purification of chloroplast DNA, mitochondrial DNA, and nuclear DNA from the tumor tissue and analysis of each for the presence of T-DNA by the Southern blotting and hybridization technique.

MATERIALS AND METHODS

Plant Tissue Cultures. The crown gall tumor line BT37 was kindly provided by Armin C. Braun. This tumor line, which was cloned from a single cell in Braun's laboratory, was incited in a Havana tobacco plant by *A. tumefaciens* strain T37. Tissue was maintained on Murashige–Skoog medium (purchased from Flow Laboratories, Rockville, MD) at 25°C under fluorescent light.

Normal Havana tobacco callus tissue was kindly provided by Fred Meins, and was maintained on Murashige–Skoog medium supplemented with naphthaleneacetic acid (1 mg/liter) and kinetin (0.1 mg/liter) at 25°C under fluorescent light.

Bacterial Strain. The source of Ti plasmid DNA used here was *A. tumefaciens* strain A208 (21), a transconjugant containing pTi T37, the tumor-inducing plasmid of strain T37. Bacteria were maintained on nutrient agar plates, and single colonies were used to inoculate cultures for plasmid DNA isolation.

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Abbreviations: Ti plasmid, tumor-inducing plasmid; NaCl/Cit, standard saline citrate.

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Isolation of Ti Plasmid DNA. Ti plasmid was isolated as described by Currier and Nester (22) and modified by Sciaky *et al.* (21). All pTi T37 plasmid DNA samples used here were fingerprinted with *Sma* I and found to have the fragmentation pattern reported for pTi T37 (21).

Isolation of Chloroplast and Mitochondrial DNA. Fresh plant tissue (teratoma or normal callus) (200 g) was minced at 0°C with razor blades in the presence of an equal weight of buffer A (23): 0.3 M mannitol/0.05 M Tris·HCl, pH 8.0/3 mM EDTA (autoclaved) adjusted to 0.1% bovine serum albumin/1 mM 2-mercaptoethanol. Tissue was minced in 20- to 25-g batches in deep petri dishes for *ca.* 10 min per batch. The homogenate was filtered through four layers of cheesecloth and one layer of Miracloth (Sigma). The green turbid filtrate was centrifuged at 100 × *g*, 4°C, for 10 min, and a small pellet was discarded. The supernate was centrifuged at 1800 × *g*, 4°C, for 15 min, and the resulting pellet was designated "chloroplast fraction" and set aside on ice. The supernate was next centrifuged at 10,000 × *g*, 4°C, for 15 min, and the resulting pellet was designated "mitochondrial fraction."

Chloroplast and mitochondrial pellets were separately resuspended in 25 ml of buffer A, adjusted to 0.013 M $MgCl_2$, and treated with DNase (Worthington DPFF DNase at 150 μg/ml in some experiments, Sigma DN25 DNase at 500 μg/ml in others) at 0°C for 1 hr with occasional swirling. (The conditions of DNase treatment of chloroplasts and mitochondria were determined empirically: when too mild a treatment is employed, plastid DNA preparations are contaminated with nuclear DNA fragments.) After termination of the DNase treatment with EDTA (pH 8.0, 0.01 M, final concentration), the chloroplast and mitochondrial pellets were recovered by centrifugation at 1800 × *g* and 10,000 × *g*, respectively, for 15 min at 4°C.

The pellets were resuspended in 10 ml of buffer A and were layered on step gradients consisting of 10 ml each of 60% and 30% (wt/vol) sucrose in 0.05 M Tris/3 mM EDTA, pH 8.0, prepared in Spinco SW 27 cellulose nitrate tubes. Gradients were centrifuged at 20,000 rpm for 15 min (at speed) in the Spinco SW 27 rotor (5°C). The green material at the interface between 30% and 60% sucrose steps (removed by pasteur pipette) was found to contain the bulk of both chloroplast and mitochondrial fractions. Material from the 60% pellet and from the step between the applied layer and 30% sucrose yielded little or no DNA.

The chloroplast and mitochondrial fractions were diluted with buffer A (30 ml) and centrifuged (1800 × *g* and 10,000 × *g*, respectively, 15 min). Pellets were resuspended in 10–20 ml of 0.15 M NaCl/0.1 M EDTA, pH 8.0, and centrifuged once more (1800 × *g* and 10,000 × *g*, 15 min).

The mitochondrial pellet was frozen (dry ice/ethanol) and thawed (22°C waterbath) three times to aid in lysis. Pellets were resuspended in 2.1 ml of TES buffer: 0.05 M Tris/0.02 M EDTA/0.15 M NaCl, pH 8.0. To each was added 0.6 ml of 10% Sarkosyl (CIBA–Geigy) and 0.3 ml of a 5 mg/ml solution of predigested Pronase (Sigma) (22). After 30-min incubation at 22°C, to each sample was added 0.6 ml of TES buffer and 3.8 g of CsCl (Merck). Samples were refrigerated for 1–3 hr. To each was added 0.25 ml of ethidium bromide (Sigma) solution (10 mg/ml). Samples were centrifuged in the Spinco SW 50.1 rotor in polyallomer tubes (washed with 10% Sarkosyl) for 3 days at 32,000 rpm. One gradient sufficed per plastid fraction from 200 g of fresh tissue.

Upon illumination with long-wavelength UV light, gradients exhibited a single sharp fluorescent band in most cases. Occasionally a faint lower band was observed that we presume was a covalently closed circular form of plastid DNA; in such cases, lower and upper bands were combined. Fluorescent bands were removed from the gradients by pasteur pipette, extracted with isopropyl alcohol saturated with 3 M NaCl until the dye was removed, and dialyzed against 10 mM Tris·HCl, pH 8.0. The DNA was extracted with phenol/0.1% 8-hydroxyquinoline (equilibrated with 3% NaCl) and exhaustively dialyzed against 10 mM Tris·HCl, pH 8.0. DNA concentration was determined by a fluorescence assay (24).

Isolation of Nuclear DNA from BT37 Crown Gall Tissue. BT37 tumor tissue (200 g) was minced by razor blades as described above in 200 ml of homogenization buffer: 1.14 M sucrose/5 mM $MgCl_2$/0.01 M Tris·HCl, pH 8.0 (autoclaved), adjusted to 6 mM 2-mercaptoethanol (25). The homogenate was filtered through cheesecloth and Miracloth. A crude nuclear pellet was collected by centrifugation at 1400 × *g* for 10 min. Nuclei were resuspended in 20 ml of Triton wash buffer (0.25 M sucrose/0.05 M Tris·HCl, pH 8.0/2 mM $MgCl_2$/2 mM $CaCl_2$/2% Triton X-100, autoclaved) (26) in a Dounce homogenizer (B pestle). Nuclei were collected by centrifugation (1400 × *g*, 10 min). The Triton wash procedure was repeated a total of five times, followed by two washes in 0.05 M Tris·HCl, pH 8.0. The pellet (*ca.* 1 g) was frozen in dry ice/ethanol and stored at −80°C.

For DNA isolation, a Dounce homogenizer containing 5 ml of 0.006 M Na_2HPO_4/0.002 M NaH_2PO_4/0.001 M EDTA/0.01 M Tris, pH 9.0/2 M NaCl, was heated to 65°C. The frozen nuclear pellet was added and quickly dispersed with the homogenizer. After 15-min incubation at 65°C, the mixture was centrifuged (12,000 × *g*, 10 min) and DNA was precipitated from the supernate by addition of 2 vol of 95% (vol/vol) ethanol. DNA was redissolved in 0.05 M Tris·HCl/0.02 M EDTA, pH 8 (4 ml) and treated with predigested Pronase (22) (500 μg/ml) for 1 hr. The preparation was centrifuged to equilibrium in a CsCl/ethidium bromide gradient (22), and DNA from the fluorescent band was further purified by dye extraction, dialysis, phenol extraction, and further dialysis as described above for plastid DNA samples. DNA concentration was determined by a fluorescence assay (24).

Restriction Enzyme Digests and Electrophoretic Separation. *Eco*RI was isolated by the procedure of Sumegi et al. (27). *Bst* I, an isoschizomer of *Bam*HI, was a gift of Richard Meagher, University of Georgia. Restriction endonuclease digestions were performed at 37°C in 0.1 M Tris·HCl, pH 7.6/0.05 M NaCl/0.01 M $MgCl_2$ (*Eco*RI) or 0.1 M Tris·HCl, pH 7.6/0.1 M NaCl/0.006 M $MgCl_2$ (*Bst* I). Digest fragments were separated by electrophoresis in 0.7% agarose gels (21). After staining and photography, the gel was used to prepare nitrocellulose membrane (Millipore HAWP 304 F) transfers by the procedure of Southern (28). Transfers were baked in a vacuum oven for 2 hr at 80°C immediately before use.

Labeling of pTi T37 Probe DNA. Ti plasmid DNA was labeled by the nick-translation procedure (29, 30) using three α-^{32}P-labeled deoxynucleoside triphosphates (New England Nuclear, 350 Ci/mmol; 1 Ci = 3.7 × 10^{10} becquerels) plus unlabeled deoxyguanosine triphosphate.

Hybridization Reactions. In the following description, 1 × NaCl/Cit is 0.15 M NaCl/0.015 M trisodium citrate (standard saline citrate), and 1 × PM is the preincubation mixture of Denhardt (31): 0.02% Ficoll/0.02% bovine serum albumin/0.02% polyvinylpyrrolidone (PVP 360, Sigma). Cellulose nitrate blots were preincubated in 6 × NaCl/Cit, 10 × PM at 68°C overnight. They were transferred to sealable plastic bags (Sears Roebuck, Seal-N-Save) and incubated at 68°C for 4 hr in 20 ml of 3 × NaCl/Cit/5 × PM/20 mM Tris·HCl, pH 7.4/2 mM EDTA/0.1% sodium dodecyl sulfate/20 μg of denatured unlabeled trout sperm DNA per ml. The sealed bag was opened

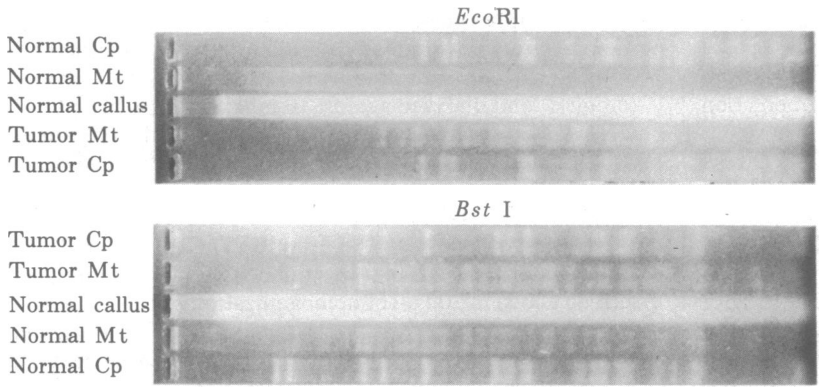

FIG. 1. Cleavage patterns of chloroplast (Cp) and mitochondrial (Mt) DNAs isolated from BT37 tobacco crown gall tumor tissue and normal tobacco callus. Plastid DNA samples (0.5 μg) were digested with *Eco*RI or *Bst* I (an isoschizomer of *Bam*HI). Normal tobacco callus DNA digests were included as hybridization controls (see Fig. 2). The digests pictured here were used to prepare the Southern blots whose autoradiograms are shown in Fig. 2.

and to the contents was added denatured ^{32}P-labeled pTi T37 probe DNA (87×10^6 cpm/μg, 0.5 μg/20 ml of final hybridization mixture). The bag was resealed and incubated at 68°C for 42 hr. Blots were rinsed with 300–500 ml of 3 × NaCl/Cit (twice, room temperature), 2.5 × NaCl/Cit/5 mM EDTA/0.2% sodium dodecyl sulfate (four times, 68°C, 30 min per wash), and 0.1 × NaCl/Cit/5 mM EDTA, pH 8.0/0.2% sodium dodecyl sulfate (once, 68°C, 15 min). Blots were subjected to autoradiography for 4 days at −80°C, with two Du Pont Cronex intensification screens. The blot was mounted on transparent plastic and, together with a sheet of X-ray film, placed between the screens during autoradiographic exposure.

RESULTS

Restriction Endonuclease Cleavage Patterns of Plastid DNAs. Chloroplast and mitochondrial DNA were isolated from tobacco crown gall teratoma line BT37 and from normal tobacco callus. The plastid DNAs were cleaved with *Eco*RI and *Bst* I, and the fragments were compared after electrophoretic separation in 0.7% agarose gels. Tumor chloroplast DNA and normal chloroplast DNA yielded identical simple cleavage patterns with both enzymes (Fig. 1). Tumor mitochondrial DNA and normal mitochondrial DNA yielded complex patterns with *Bst* I that exhibit no clear differences. Digestion of normal mitochondrial DNA with *Eco*RI was unsuccessful; hence comparison with this enzyme was not possible.

Analysis of Plastid and Nuclear DNAs for Presence of T-DNA. Five micrograms of tumor nuclear DNA, 0.5 μg of tumor chloroplast DNA, and 0.5 μg of tumor mitochondrial DNA as well as control DNA samples [normal chloroplast DNA, 0.5 μg; normal mitochondrial DNA, 0.5 μg; and total normal tobacco callus DNA isolated as described (7), 5 μg] were cleaved with *Eco*RI or *Bst* I. Digests were fractionated by electrophoresis and the fragments were blotted onto nitrocellulose by the Southern procedure (28). For detection of T-DNA fragments, we employed as hybridization probe ^{32}P-labeled pTi T37 DNA, the Ti plasmid of the *A. tumefaciens* strain that incited the tumor line under investigation.

As expected, the labeled Ti plasmid did not hybridize detectably to fragments of normal callus total DNA, normal callus chloroplast DNA, or normal callus mitochondrial DNA (Fig. 2). Bands of hybridization are readily visible in autoradiograms of both the *Eco*RI and *Bst* I digests of BT37 tumor nuclear DNA, but not in those of the digests of tumor chloroplast or

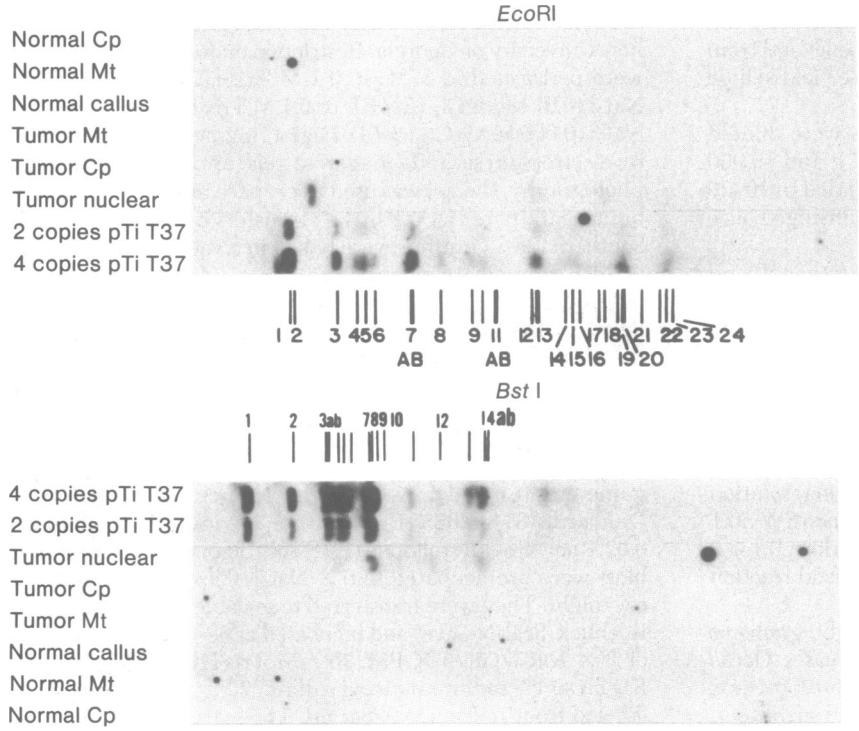

FIG. 2. Autoradiograms of *Eco*RI and *Bst* I digest fragments hybridized with labeled pTi T37 DNA. *Eco*RI and *Bst* I digests of the following DNAs were each separated by horizontal agarose slab gel electrophoresis: normal tobacco callus chloroplast (Cp) DNA, 0.5 μg; normal tobacco callus mitochondrial (Mt) DNA, 0.5 μg; total DNA isolated as described (7) from normal tobacco callus, 5 μg; BT37 tumor Cp DNA, 0.5 μg; BT37 tumor Mt DNA, 0.5 μg; nuclear DNA isolated from BT37 tumor, 5 μg; and reconstruction mixtures containing 5 μg of salmon DNA plus 0.2 ng (2 copies) or 0.4 ng (4 copies) of pTi T37 DNA. The reconstructions mimic 5 μg of tobacco DNA containing 2 or 4 copies of the entire Ti plasmid per diploid cell, as calculated previously (7). Southern blots were prepared from the gel (28) and hybridized with ^{32}P-labeled pTi T37 DNA. A line drawing of the cleavage pattern of pTi T37 with *Eco*RI is presented below the autoradiogram; the drawing for *Bst* I is presented above its autoradiogram.

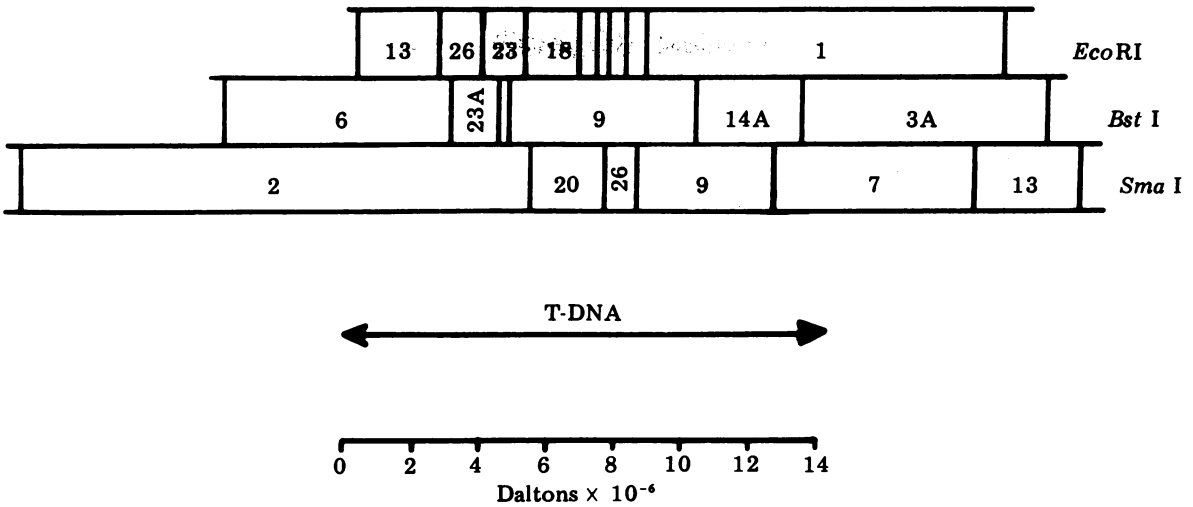

FIG. 3. Map of the T-DNA region of pTi T37. Two types of evidence (to be published separately) were used to determine the *Sma* I fragment map shown here: (*i*) Fingerprints of a series of deletion mutants of pTi T37 extending in from the left edge of T-DNA; and (*ii*) hybridization of purified labeled *Hpa* I and *Bst*EII fragments of pTi T37 to Southern blots of *Sma* I-digested pTi T37. *Bam*HI (an isoschizomer of *Bst* I) fragments of pTi T37 were cloned in pBR322 under P2 containment in EK1 strains of *Escherichia coli*. Clones hybridizing to *Sma* I fragments in the T-DNA region were analyzed. Clones of fragments 17 + 6 (a partial digestion product), 23A + i + 9 (in which i represents a small fragment beyond the numbered fragments on the gel) (also a partial digestion product), 14A, and 3A were cleaved with *Bam*HI, *Sma* I, and *Eco*RI in all possible single- and double-digestion combinations. From the analysis of molecular weights of single- and double-digestion products, the order of the *Eco*RI fragments was established. Details of the mapping of pTi T37 restriction endonuclease fragments will be presented elsewhere.

tumor mitochondrial DNA. As an indication of the sensitivity of the hybridization reaction, two reconstruction mixtures were included in the study. These showed that as little as 2 copies of the Ti plasmid per diploid tobacco cell DNA equivalent are detectable under our experimental conditions. The T-DNA fragments detected in the nuclear DNA samples appear by inspection to represent 2–4 copies per cell.

The portion of pTi T37 that is detected in the BT37 tobacco tumor line analyzed here extends over about 21 kilobases (14×10^6 daltons) and contains several *Eco*RI and *Bst* I cleavage sites. The fragments of BT37 tumor nuclear DNA that hybridize with labeled Ti plasmid DNA probe should therefore include recognizable "internal" *Eco*RI or *Bst* I fragments of Ti plasmid DNA. In addition, if T-DNA is attached to plant DNA, there should exist "border fragments" with only partial homology to the probe. If two or more T-DNA copies are arranged in tandem, there would also exist "fusion fragments," fully homologous to the probe. "Border fragments" and "fusion fragments" could be of any molecular weight, but could be distinguished by their intensities: "border fragments" should appear relatively faint in the autoradiogram because they contain nonhybridizing plant DNA sequences; "fusion fragments" should appear as bright as "internal" fragments. Moreover, fusion fragments should hybridize with probes from noncontiguous parts of the Ti plasmid (i.e., from the edges that are fused).

Fig. 3 is a map of the known *Bst* I and *Eco*RI restriction endonuclease cleavage sites in pTi T37 in the region of T-DNA. Line drawings of the cleavage patterns of pTi T37 with *Eco*RI and *Bst* I are presented adjacent to the autoradiograms of Fig. 2 to allow assignment of fragment numbers. The most strongly hybridizing and largest molecular weight fragment of T-DNA in the *Eco*RI digest of tumor nuclear DNA (Fig. 2) does not correspond to any *Eco*RI fragment of the original Ti plasmid: it hybridizes with labeled probes from the left and right edges of T-DNA [*Bst* I fragments 14A (32) and 6 (data not shown) as well as cloned *Hin*dIII fragments from the left and right borders of T-DNA (33)]. This large T-DNA fragment is therefore a "fusion fragment" and indicates a tandem arrangement of at least some T-DNA copies. Detailed analysis of this fragment, isolated from tumor DNA by inserting into Charon 4A bacteriophage and cloning, has confirmed directly that it contains left and right ends of T-DNA, and in addition some DNA that was not in the original Ti plasmid (33). Nine smaller *Eco*RI fragments of T-DNA have been detected in this tumor line (33), of which only the four largest are clearly visible in Fig. 2. Eight of these smaller fragments of T-DNA have been cloned in Charon 4A bacteriophage in this laboratory and elsewhere (33); a detailed analysis of seven of these fragments will be presented separately.

The T-DNA fragments visible in the *Bst* I digest of tumor nuclear DNA (Fig. 2) include three that comigrate with the expected Ti plasmid fragments in the T-DNA region (*Bst* I fragments 9, 14A, and 23A). There are in addition three faint presumptive border fragments. A detailed analysis of these fragments will be presented separately. Our data support the view that there are at least two separate inserts, and that some ends of inserts occur in the middle of T-DNA copies (by "copy" we mean the apparent stretch of T-DNA on the Ti plasmid map).

DISCUSSION

T-DNA fragments are detected readily in 5 μg of nuclear DNA from tobacco crown gall teratoma BT37 in the Southern blot hybridization presented here; they are not detectable in 0.5 μg of pure chloroplast or mitochondrial DNA from this tumor line, analyzed concurrently. The digest of our "nuclear" DNA, like that of total tumor DNA, contains faintly visible bands that match the positions of chloroplast DNA digest fragments (data not shown); mitochondrial DNA bands are not visible. By this visual criterion, our "nuclear" DNA sample cannot be so grossly contaminated with chloroplast or mitochondrial DNA that 5 μg of it contains more than 0.5 μg of either kind of plastid DNA. The T-DNA fragments detected in nuclear DNA are therefore not ascribable to contaminating plastid DNA, but to nuclear DNA itself. These experiments do not rigorously rule out the possibility that T-DNA is of extranuclear origin, and (like chloroplast DNA) is coisolated with the nuclear fraction.

The finding that T-DNA appears in the nuclear DNA fraction raises the possibility that T-DNA may be covalently joined

to chromosomal DNA. The appearance of low-intensity "border fragments" in our analysis of tumor DNA digests is consistent with this view. These "border fragments" vary in size in different tumor lines (unpublished data). The analysis of such "border fragments" of T-DNA by molecular cloning techniques will yield definitive evidence on this issue.

The fact that T-DNA is not associated with the known extrachromosomal DNAs of the plant cell, the plastid DNAs, rules out one possible explanation of the loss of T-DNA (9) during recovery of normal plants from crown gall tumor cells by grafting (19, 20). The loss of T-DNA cannot be ascribed to segregation of affected plastids. If T-DNA is indeed attached to nuclear DNA, the loss of T-DNA during recovery must be due to one or several deletion events, which might also affect flanking plant DNA.

The nuclear location of T-DNA would indicate that transcription of this foreign DNA of prokaryotic origin is effected by nuclear RNA polymerase in the plant cell. The nature of the promoters and the possible presence of intervening sequences in the transcripts are thus of great interest. The important issue of whether T-DNA is covalently joined to plant chromosomal DNA or functioning as an independent plant-DNA-containing replicon remains to resolved. The location of T-DNA in the nuclear DNA fraction of the tumor cell is an encouraging finding for those wishing to employ T-DNA as a vector for introducing novel DNA into higher plant cells.

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1. Zaenen, I., Van Larebeke, N., Teuchy, H., Van Montagu, M. & Schell, J. (1974) *J. Mol. Biol.* **86**, 109–127.
2. Van Larebeke, N., Engler, G., Holsters, M., Van den Elsacker, S., Zaenen, I., Schilperoort, R. A. & Schell, J. (1974) *Nature (London)* **252**, 169–170.
3. Watson, B., Currier, T. C., Gordon, M. P., Chilton, M.-D. & Nester, E. W. (1975) *J. Bacteriol.* **123**, 255–264.
4. Petit, A., Delhaye, S., Tempé, J. & Morel, G. (1970) *Physiol. Veg.* **8**, 205–213.
5. Bomhoff, G., Klapwijk, P. M., Kester, H. C. M., Schilperoort, R. A., Hernalsteens, J. P. & Schell, J. (1976) *Mol. Gen. Genet.* **145**, 177–181.
6. Montoya, A. L., Chilton, M.-D., Gordon, M. P., Sciaky, D. & Nester, E. W. (1977) *J. Bacteriol.* **129**, 101–107.
7. Chilton, M.-D., Drummond, M. H., Merlo, D. J., Sciaky, D., Montoya, A. L., Gordon, M. P. & Nester, E. W. (1977) *Cell* **11**, 263–271.
8. Merlo, D. J., Nutter, R. C., Montoya, A. L., Garfinkel, D. J., Drummond, M. H., Chilton, M.-D., Gordon, M. P. & Nester, E. W. (1980) *Mol. Gen. Genet.* **177**, 637–643.
9. Yang, F.-M., Montoya, A. L., Merlo, D. J., Drummond, M. H., Chilton, M.-D., Nester, E. W. & Gordon, M. P. (1980) *Mol. Gen. Genet.* **177**, 707–714.
10. Van Montagu, M. & Schell, J. (1979) in *Plasmids of Bacteria of Medical, Environmental and Commercial Importance*, ed. Timmis, K. (Elsevier/North-Holland, Amsterdam), pp. 71–96.
11. Thomashow, M. F., Nutter, R., Montoya, A. L., Gordon, M. P. & Nester, E. W. (1980) *Cell* **19**, 729–739.
12. Kleckner, N. (1977) *Cell* **11**, 11–23.
13. Drummond, M. H., Gordon, M. P., Nester, E. W. & Chilton, M.-D. (1977) *Nature (London)* **269**, 535–536.
14. Ledeboer, A. M. (1978) Dissertation (University of Leiden, Leiden, The Netherlands).
15. Gurley, W. B., Kemp, J. D., Albert, M. J., Sutton, D. W. & Callis, J. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 2828–2832.
16. Chilton, M.-D., McPherson, J., Saiki, R. K., Thomashow, M. F., Nutter, R. C., Gelvin, S. B., Montoya, A. L., Merlo, D. J., Yang, F.-M., Garfinkel, D. J., Nester, E. W. & Gordon, M. P. (1979) in *Emergent Techniques for the Genetic Modification of Crop Plants*, ed. Rubenstein, I. (Univ. of Minnesota Press, Minneapolis, MN), in press.
17. Kung, S. (1977) *Annu. Rev. Plant Physiol.* **28**, 401–437.
18. Quétier, F. & Vedel, F. (1977) *Nature (London)* **268**, 365–368.
19. Braun, A. C. & Wood, H. N. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 496–500.
20. Turgeon, R., Wood, H. N. & Braun, A. C. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 3562–3564.
21. Sciaky, D., Montoya, A. L. & Chilton, M.-D. (1978) *Plasmid* **1**, 238–253.
22. Currier, T. C. & Nester, E. W. (1976) *Anal. Biochem.* **76**, 431–441.
23. Kolodner, R. & Tewari, K. K. (1975) *Biochim. Biophys. Acta* **402**, 372–390.
24. Le Pecq, J.-B. (1971) in *Methods of Biochemical Analysis*, ed. Glick, D. (Interscience, New York), Vol. 20, pp. 41–86.
25. Hamilton, R. H., Kunsch, U. & Temperli, A. (1972) *Anal. Biochem.* **49**, 48–57.
26. Hendricks, A. W. (1972) *FEBS Lett.* **24**, 101–105.
27. Sumegi, J., Breedveld, D., Hossenlopp, P. & Chambon, P. (1977) *Biochem. Biophys. Res. Commun.* **76**, 78–85.
28. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
29. Kelly, R. B., Cozzarelli, N. R., Deutscher, M. P., Lehman, I. R. & Kornberg, A. (1970) *J. Biol. Chem.* **245**, 39–45.
30. Maniatis, T., Jeffrey, A. & Kleid, D. G. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 1184–1188.
31. Denhardt, D. T. (1966) *Biochem. Biophys. Res. Commun.* **23**, 641–646.
32. Chilton, M.-D., Yang, F.-M., Saiki, R. K., Postle, K., Montoya, A. L., Nester, E. W., Quétier, F. & Gordon, M. P. (1979) in *Genome Organization and Expression in Plants*, ed. Leaver, C. J. (Plenum, New York), pp. 471–480.
33. Schell, J. & Van Montagu, M. (1979) in *Genome Organization and Expression in Plants*, ed. Leaver, C. J. (Plenum, New York), pp. 453–470.