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# Transposon Tn*5* mediated gene transfer into plants

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**Summary.** A novel bacterial transposon, Tn*5-PV*, that can used for mapping and analysis of the expression of genes transferred into plant cells, was constructed by insertion of a plant gene vector into Tn*5* without alteration of its transposition properties. Tn*5-PV* carries $ori_V$ and $ori_T$ sequences of the broad host range plasmid RK2 linked to selectable and screenable plant marker genes and to the 25 bp border sequences of *Agrobacterium* Ti plasmid T-DNAs, orientated towards the ends (IS50L and IS50R) of Tn*5*. Plasmids with Tn*5-PV* insertions are converted by the transposon insertion into plant gene vectors that can be efficiently mobilized from *Escherichia coli* and stably maintained in *Agrobacterium* hosts. Due to the orientation of the T-DNA borders, Tn*5-PV* mediates the transfer of target plasmids from *Agrobacterium* to plant cells. The usefulness of this approach was demonstrated by transferring independent transposon insertions in T-DNA tumour genes to tobacco cells. The correlation between the map position of transposon insertions, tumour phenotypes, the absence of specific T-DNA transcripts and the structural analysis of pGV354::Tn*5-PV* derivatives in DNA from tobacco tumours, showed that T-DNA genes linked to the chimeric plant marker genes of Tn*5-PV* were transferred without any rearrangement into plant cells.

**Key words:** Transposon Tn*5* mutagenesis – Genetransfer to plants – *Agrobacterium* binary vector system

## Introduction

The bacterial transposon Tn*5* is a valuable tool for the isolation, mapping and cloning of gene mutations in prokaryotic organisms (Berg and Berg 1983; de Bruijn and Lupski 1984). A great number of Tn*5* derivatives have been constructed to elucidate the mechanisms of Tn*5* transposition (Berg et al. 1980) and to provide Tn*5* with different bacterial selectable markers (Zsebo et al. 1984; Rella et al. 1985). Tn*5* derivatives have also been used as tools to probe bacterial promoters (Kroos and Kaiser 1984; Bellofatto et al. 1984), to isolate protein export signal sequences (Manoil and Beckwith 1985) and to facilitate the conjugational transfer of chromosomes and plasmids and the isolation of genes by transposon gene tagging. Similarly, many alternative methods have been developed to deliver transposon Tn*5* efficiently into various Gram-negative bacteria using either suicide plasmids or bacteriophages (Kuner and Kaiser 1981; Beringer et al. 1978; Shanabruch et al. 1981; Selvayar and Iyer 1983; Simon et al. 1983; Laird and Young 1980).

The goal of the present work was to combine plant gene transfer with transposon Tn*5* mutagenesis techniques. A new transposon, Tn*5-PV*, sharing traits of transposon Tn*5* and binary plant vectors derived from the *Agrobacterium* Ti plasmid (Koncz and Schell 1986) was constructed. Insertion of a plant gene vector in the central position of Tn*5* provided Tn*5-PV* with the $ori_V$ and $ori_T$ sequences of plasmid RK2 and thus with replication and mobilization functions (Thomas et al. 1984; Guiney and Yakobson 1983). Plasmids with a transposon Tn*5-PV* insertion can be mobilized back and forth between *E. coli* and *Agrobacterium* hosts containing RK2 plasmid replication and mobilization helper functions (Koncz and Schell 1986). In Tn*5-PV* the $ori_V$ and $ori_T$ sequences are flanked by 25 bp sequences derived from the T-DNA borders of *Agrobacterium* Ti plasmids (Wang et al. 1984). During *Agrobacterium*–plant cell interaction these border sequences are recognized by the products of the induced virulence loci of the Ti plasmid as boundaries of a DNA unit to be transferred into plant cells (Stachel et al. 1985, 1986). Due to the orientation of the T-DNA borders relative to the IS50L and IS50R ends of Tn*5-PV*, insertion of Tn*5-PV* in a plasmid will result in a construction that can be transferred to plants in its entirity. As a consequence, cloned genes harboured by any plasmid can be transferred (or inactivated and transferred) to plant cells by Tn*5-PV*. After isolation of random Tn*5-PV* insertions in a large cloned DNA sequence, coding regions can be mapped by analysis of the resulting transcripts in transformed plants. Genes not affected by transposon insertion can be simultaneously identified and their level of expression can be compared to that of co-transferred chimeric plant marker genes of Tn*5-PV* used as standards. The application of this approach is demonstrated using a model plasmid, pGV354, which carries the internal T-DNA genes (1, 2, 4, 5) of pTiC58 (Willmitzer et al. 1983; Joos et al. 1983).

## Materials and methods

*Bacterial strains and plasmids* used are listed in Table 1.

*Microbiological and cloning methods.* Bacterial media, culture conditions, concentrations of antibiotics, isolation of

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**Table 1.** Bacterial strains and plasmids

| Bacterial strains | Relevant markers | Reference |
|---|---|---|
| *Escherichia coli* | | |
| CA 8000 | CSH62, thi, Hfr | Miller (1982) |
| HB101 | *recA, pro, leu, lacY, supF* $Sm^r\ r_K^-m_K^-, F^-$ | Boyer and Roulland-Dussoix (1969) |
| HB101 $Rif^r$ | as HB101, $Rif^r$ | A. Kondorosi |
| SM10 | *thi thr leu* $su_{III}$ | Simon et al. (1983) |
| *Agrobacterium tumefaciens* | | |
| GV3101 | $Rif^r$ | Van Larebeke et al. (1974) |
| GV3103 | $Sm^r\ Sp^r$ | Holsters et al. (1980) |
| *Plasmids* | | |
| pGV748::Tn*5* | $Ap^r\ Cm^r$ Sm/$Sp^r$::Tn*5* | A. Caplan |
| pPCV123 | $Ap^r\ Cm^r$ | Koncz and Schell (1986) |
| pGV354 | $Ap^r$ | Depicker et al. (1980) |
| pMP90 | $Gm^r$ | |
| pMP90RK | $Gm^r\ Km^r$ | Koncz and Schell (1986) |

bacterial plasmid and chromosomal DNAs were as described previously (Koncz et al. 1983, 1984; Koncz and Schell 1986). Cloning and physical mapping procedures were according to methods described by Maniatis et al. (1982).

*Construction and characterization of transposon Tn*5-PV. A transposon Tn*5* insertion in plasmid pGV748 was kindly provided by Dr. A. Caplan (Laboratory of Genetics, AKG-Belgium). The construction of binary *Agrobacterium* vectors containing plant gene vector cassettes, based on a conditional mini-RK2 replicon linked to T-DNA border repeats of *Agrobacterium* Ti plasmids and to chimeric neomycin phosphotransferase and octopine synthase genes, has been described previously (Koncz and Schell 1986). The construction of Tn*5-PV* is shown in Fig. 1. The *Bam*HI fragment of the binary vector pPCV123, which carries the mini-RK2 plant gene vector, was isolated and inserted into the central *Bam*HI site of Tn*5* in plasmid pGV748::Tn*5*. A recombinant plasmid pGV748::Tn*5-PV* was obtained by selection for $Km^rCm^r$ *E. coli* transformants. The plasmid DNA from one such transformant was isolated, characterized and transformed to Hfr *E. coli* strain CA8000. Chromosomally integrated Tn*5-PV* copies were isolated by conjugation of this Hfr donor strain with *recA*, $F^-$ *E. coli* strain HB101 $Rif^r$ according to Caplan et al. (1985). After conjugation for 90 min at 37°C in liquid, $Km^r$ transconjugants resulting from Tn*5-PV* transposition from donor DNA to the recipient chromosome were obtained with a frequency of $0.5–2.5 \times 10^{-5}$. This transposition frequency is similar to that observed for Tn*5* itself in the control experiments, thus indicating that the insertion in Tn*5-PV* did not affect its transposition capacity.

*Tn*5-PV *mutagenesis in* E. coli *and transfer of plasmids with transposon insertions to Agrobacterium.* pGV354 was transformed into an *E. coli* transposon donor strain (HB101 $Rif^r$ chrX::Tn*5-PV*). In order to select Tn*5-PV* insertions in pGV354, a purified transformant was grown to late logarithmic phase and plated on neomycin gradient plates according to Simon (1984). Tn*5-PV* inserted in a multicopy plasmid should express a higher level of neomycin resistance than when present as a single copy in the chromosome. Therefore colonies growing near the centre of plates were picked purified and used for mini-scale isolation of plasmid DNAs. Mapping of the Tn*5-PV* insertions in plasmid pGV354::Tn*5-PV* derivatives was performed with *Hin*dIII, *Kpn*I, *Sal*I, *Xba*I, *Eco*RI, *Xho*I, *Sma*I, *Pst*I and *Hpa*I restriction endonucleases. pGV354::Tn*5-PV* derivatives were transferred by conjugation from *E. coli* strain SM10 to *Agrobacterium* host GV3101 (pMP90RK) as described previously (Koncz and Schell 1986). *Agrobacterium* transconjugants were selected on the basis of their $Rif^rKm^rCb^r$ phenotype and purified.

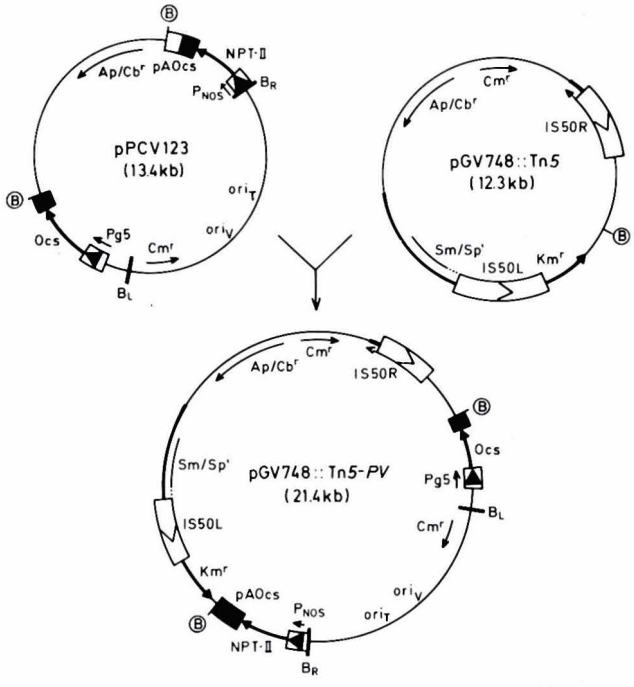


**Fig. 1.** Construction of transposon Tn*5-PV*. The *Bam*HI fragment of plasmid pPCV123 DNA was gel purified and ligated to the single *Bam*HI site of Tn*5* carried by plasmid pGV748. Ap/$Cb^r$, ampicillin/carbenicillin resistance; $Cm^r$, chloramphenicol resistance; $Km^r$, kanamycin resistance gene of Tn*5* (identical to NPTII); Sm/Sp′, streptomycin/spectinomycin resistance gene of R702 inactivated by Tn*5* insertion; Ocs, octopine synthase gene; Pg5, promoter of $T_L$-DNA encoded gene 5; $B_L$, $B_R$ left and right border sequences of Ti plasmid T-DNAs; $ori_V$, $ori_T$, replication and conjugational transfer origin sequences of plasmid RK2; $P_{NOS}$ the promoter of nopaline synthase gene; NPTII, neomycin phosphotransferase gene; pAOcs, polyadenylation signal sequence of the octopine synthase gene; B, *Bam*HI

Agrobacterium host GV3101 (pMP90RK) carries a C58 Ti plasmid derivative, the T-DNA region of which has been removed by deletion. pMP90RK also provides RK2 plasmid replication and mobilization functions and Ti plasmid virulence functions to maintain pGV354::Tn*5-PV* derivatives and to transfer their T-DNA from *Agrobacterium* into plant cells (Koncz and Schell 1986).

In order to test the stability of pGV354::Tn*5-PV* derivatives in *Agrobacterium*, each plasmid was retransferred by conjugation from *Agrobacterium* to *E. coli* HB101. Plasmid DNA was purified from 12 transconjugants of each derivative and their map was compared to that of original isolates

by restriction endonuclease digestion. Tn*5-PV* insertions in pGV354 proved to be stable in *Agrobacterium* since there was only one case out of 240 analysed where excision of Tn*5-PV* from pGV354 had occurred during the reisolation of plasmids.

To measure the transposition frequency of Tn*5-PV* in *Agrobacterium*, pGV748::Tn*5-PV* was conjugated to *Agrobacterium* strains GV3101, GV3101 (pMP90) and GV3101 (pMP90RK), from *E. coli* strain SM10. Strain GV3101 (pMP90) is isogenic with GV3101 (pMP90RK) except that it does not carry RK2 regions providing helper functions for maintainance of the mini-RK2 replicon inserted into Tn*5-PV*. Strain GV3101 is cured of its Ti plasmid. As described earlier (Koncz and Schell 1986), pGV748::Tn*5-PV* was mobilized to *Agrobacterium* GV3101 (pMP90RK) with frequencies of $0.5–1.0 \times 10^{-2}$. In strain GV3101 (pMP90) transfer and integration of plasmid pGV748::Tn*5-PV* cointegrates occurred at an average frequency of $1.0 \times 10^{-6}$, while integration of Tn*5-PV* was found at a frequency of $1.5 \times 10^{-7}$. Interestingly, significantly lower frequencies of both cointegrate and Tn*5-PV* transposition were observed after conjugating pGV748::Tn*5-PV* into *Agrobacterium* strain GV3101 ($1.8 \times 10^{-7}$ for cointegrate- and $2.8 \times 10^{-8}$ for Tn*5-PV* transposition). This indicated that Tn*5-PV* preferentially integrated in the Ti plasmid of *Agrobacterium* strain GV3101 (pMP90). Analysis of the location of Tn*5-PV* insertions by conjugating pMP90 to *Agrobacterium* recipient GV3103 showed that 68%–97% of the Tn*5-PV* insertions were indeed linked to the Ti plasmid in *Agrobacterium*.

The transposition of Tn*5-PV* in *Agrobacterium* does not affect the use of our approach since plasmid pGV354 without Tn*5-PV* cannot replicate in *Agrobacterium*. On the other hand, cells carrying only a chromosomally integrated Tn*5-PV* transposon are lost during the selection for $Rif^r Cb^r$ transconjugants.

*Transfer of plasmid::Tn*5-PV *derivatives from Agrobacterium to plants. Agrobacterium* strains harbouring plasmid pGV354::Tn*5-PV* derivatives were used for infection of *Nicotiana tabacum* (SR1) and *Kalanchoe daigremontiana* leaf discs, stem segments and plants. Tumour induction on kalanchoe and tobacco plants and tissue culture conditions have been described previously (Marton et al. 1982; DeBlock et al. 1984; Koncz et al. 1983, 1984). Tobacco leaf disc infections were carried out according to Horsch et al. (1985). Tumour tissues were maintained on hormone-free media containing 100 μg/ml kanamycin sulphate. *Agrobacteria* were counter-selected using 500 μg/ml claforan. In the case of shooter mutants the callus medium was supplemented with 1.0 mg/l NAA (naphthalene-1-aceticacid) while rooting tumours were maintained on media containing 0.5 mg/l BAP (6-benzylaminopurine).

*Plant DNA and RNA purification, hybridization conditions, octopine synthase and neomycin phosphotransferase assays.* DNA from tumour calli was purified according to Taylor and Powell (1983). Poly(A)$^+$ RNA was prepared as described by Willmitzer et al. (1982). Southern and Northern blots were prepared according to Maniatis et al. (1982) and hybridized as described previously (Koncz and Schell 1986).

Octopine synthase and neomycine phosphotransferase activities were assayed according to Otten and Schilperoort (1978) and to Schreier et al. (1985), respectively, using standardized conditions described earlier (Koncz and Schell 1986).

## Results

In order to show that the use of a bacterial transposon for mutagenesis, transfer, mapping and analysis of the transcription of genes expressed in plants is feasible and reliable we choose a well characterized model system with genes whose location, function and expression had already been studied in detail. Plasmid pGV354 (Depicker et al. 1980) carries T-DNA encoded genes of pTiC58 responsible for synthesis of auxin (genes 1 and 2), cytokinin (gene 4) and phosphorylated sugars (*Acs* gene) and gene 5, the function of which is not yet understood (Willimitzer et al. 1983; Joos et al. 1983). Due to the expression of genes 1, 2 and 4, transformed cells form tumours, while a mutation destroying the function of the auxin or cytokinin genes results in shooting or rooting tumour phenotypes, respectively (for review see Schell 1986).

The general scheme of Tn*5-PV* mediated mutagenesis and gene transfer is shown in Fig. 2. An *E. coli* strain with a chromosomally located Tn*5-PV* was used to produce transposon insertions in pGV354 as described in Materials and methods. Of 200 independent isolates, 11 Tn*5-PV* insertions were analysed and mapped. The location of these inserts is shown on a linear map of pGV354 in Fig. 3 along with the physical map of Tn*5-PV*. These inserts were introduced into *E. coli* strain SM10 and the SM10 (pGV354::Tn*5-PV*) derivatives were used to transfer the transposon – tagged pGV354 plasmids to *Agrobacterium* host GV3101 (pMP90RK).

*Transfer and analysis of the structure and expression of pGV354::Tn*5-PV *derivatives in tobacco tumour tissues*

*Agrobacterium* strains harbouring pGV354::Tn*5-PV* derivatives were used to induce tumours on kalanchoe and tobacco stems and on tobacco leaf discs. Tumours induced on both plants by derivatives 5.14, 5.18, 5.12, 5.1, 5.19, 5.20, 5.4 and 5.8 had a phenotype similar to that induced by wild-type T-DNAs. Derivatives 5.3 and 5.5, which carry a Tn*5-PV* insert in gene 1, only produced very attenuated tumours on both kalanchoe and tobacco. When transformed tobacco tissue was transferred to tissue culture medium without growth hormones this resulted in the formation of teratoma cultures. Derivative 5.2, in which gene 4 was inactivated by a Tn*5-PV* insertion, initiated excessive root formation by transformed tissues maintained in tissue culture. The Tn*5-PV* encoded screenable marker, octopine synthase, was detected in each tumour line by the appropriate enzyme assay (Fig. 4c). The tumour tissues could be maintained on media containing 100 μg/ml kanamycin. The expression of the second Tn*5-PV* encoded marker gene was confirmed by neomycin phosphotransferase assays (data not shown).

The stability of tumour phenotypes and the continuous presence of Tn*5-PV* encoded marker enzymes in the transformed tissues suggested that pGV354::Tn*5-PV* derivatives were transferred to plant cells without rearrangements and were maintained stably. In order to confirm this assumption, DNA from three randomly selected tumour lines (5.2, 5.1 and 5.14) was purified, digested with *Hin*dIII endonuclease and analysed by Southern gel hybridization using puri-

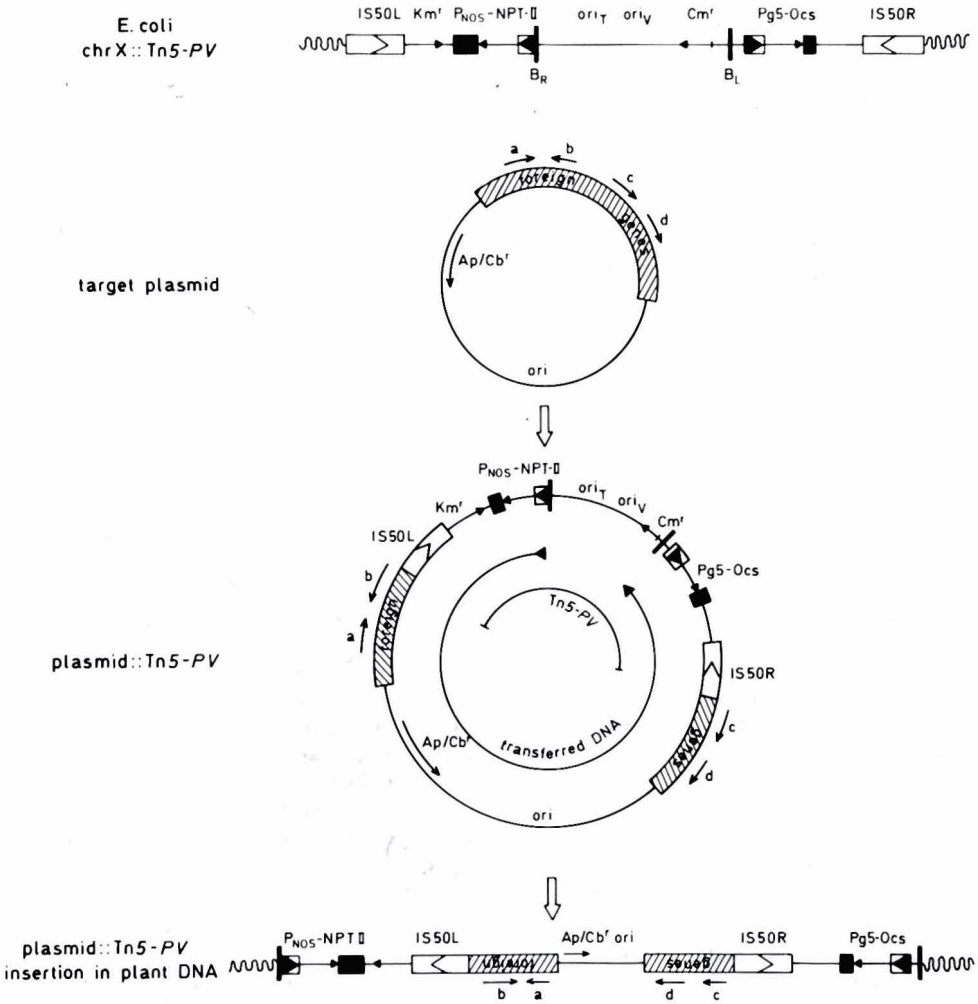


**Fig. 2.** General scheme of Tn*5-PV* mutagenesis and gene transfer. Tn*5-PV* insertions were isolated in a target plasmid using an *E. coli* donor strain (chrX::Tn*5-PV*). A structure similar to *Agrobacterium* binary plant vectors was formed by Tn*5-PV* insertion. The target plasmid linked to Tn*5-PV* was transferred into plant cell and integrated into the nuclear genome. *a, b, c, d* are putative coding sequences in a foreign DNA cloned on the target plasmid. Inversion of *a, b, c, d* sequences occurs during the integration of plasmid::Tn*5-PV* derivatives into plant DNA. Abbreviations are as Fig. 1

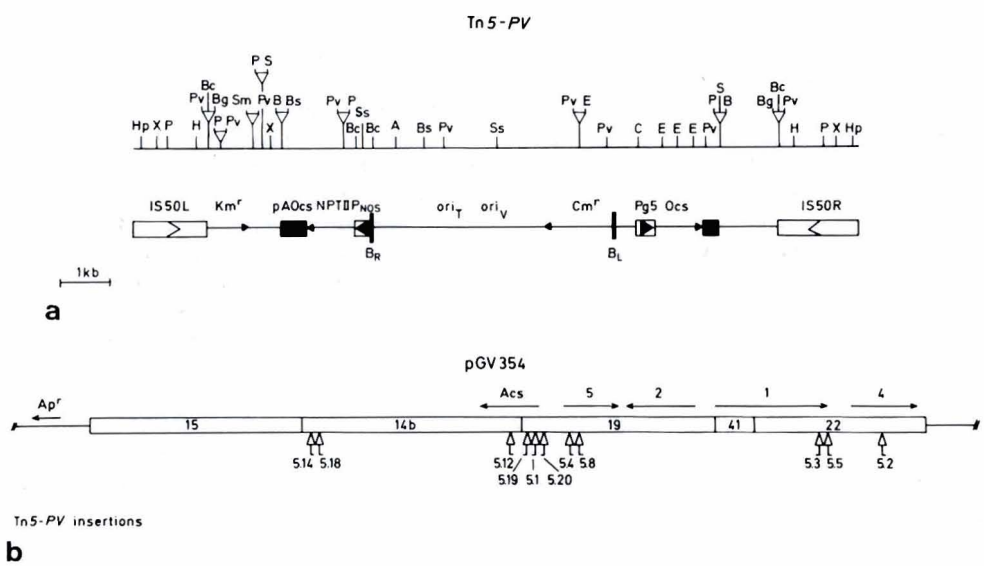


**Fig. 3. a** Physical map of Tn*5-PV*. Restriction endonuclease cleavage sites: Hp, *Hpa*I; X, *Xho*I; P, *Pst*I; H, *Hin*dIII; Pv, *Pvu*II; Bc, *Bcl*I; Bg, *Bgl*II; Sm, *Sma*I; S, *Sal*I; B, *Bam*HI; Bs, *Bst*EII; Ss, *Sst*II; A, *Apa*I; E, *Eco*RI; C, *Cla*I. The orientation of Tn*5-PV* can easily be determined using *Sma*I, *Apa*I or *Eco*RI, while its precise location can be mapped by *Hpa*I, *Xho*I or *Pst*I digestions along with pGV748::Tn*5-PV* DNA digested with the same enzymes, as a control. **b** Localization of Tn*5-PV* insertions in *Hin*dIII fragments of pGV354. The orientation of Tn*5-PV* insertions is indicated by *arrow headed L* signs and corresponds to the orientation of the octopine synthase transcription unit of Tn*5-PV*

fied *Hin*dIII fragments (15, 14b, 19 and 22) of pGV354 T-DNA and an *Eco*RI-*Hin*dIII fragment carrying IS50R of pGV748::Tn*5* DNA, as probes.

As shown in Figs. 4a and 5 the integration of pGV354::Tn*5-PV* sequences into plant DNA resulted in the expected DNA rearrangements. As a result of Tn*5-PV* insertions the split *Hin*dIII fragments of the T-DNA in pGV354 hybridized to the homologous fragment probes and to the IS50 sequences of Tn*5-PV* (see hybridization of 5.2 and 5.1 DNAs to probes *Hin*dIII 22, 19 and IS50

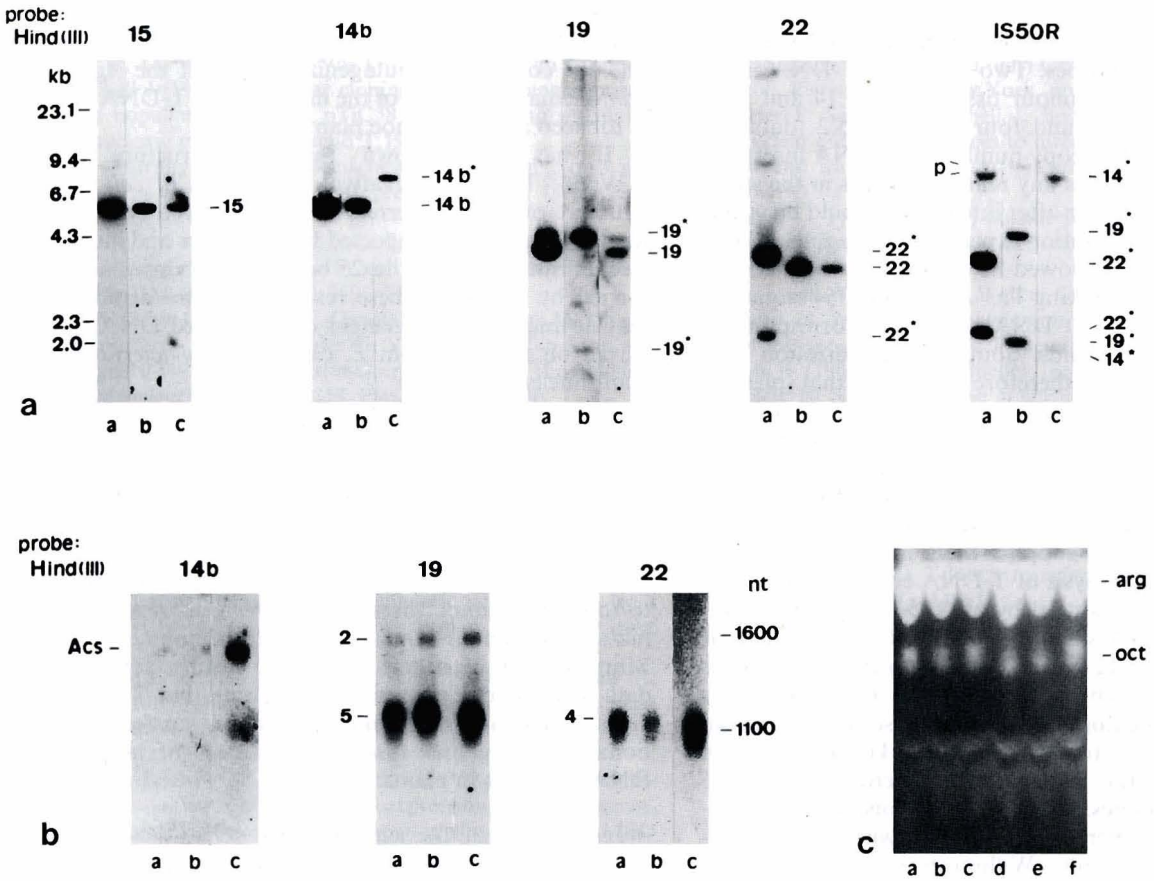


**Fig. 4.** **a** Southern gel hybridization of *Hin*dIII digested tumour DNAs containing transferable DNA inserts of pGV354::Tn*5-PV* derivatives 5.2, 5.1 and 5.14 to probes of gel-purified T-DNA *Hin*dIII fragments 15, 14b, 19 and 22 and the *Eco*RI-*Hin*dIII fragment of pGV748::Tn*5* DNA. Tumour DNAs: 5.2, (*a*); 5.1, (*b*) and 5.14, (*c*). Fragments marked by *asterisks* correspond to junction fragments between Tn*5-PV* and pGV354. P, partially digested fragments. **b** Northern gel hybridization of poly(A)$^+$ RNA samples isolated from tumour lines 5.12, (*a*); 5.1, (*b*) and 5.14, (*c*) to T-DNA *Hin*dIII fragments 14b, 19 and 22. nt, size of transcripts. **c** Paper electrophoretic detection of octopine synthase enzyme activities in tumour lines 5.1, (*a*); 5.3, (*b*); 5.4, (*c*); 5.5, (*d*); 5.8, (*e*); 5.14, (*f*). arg, arginine; oct, octopine

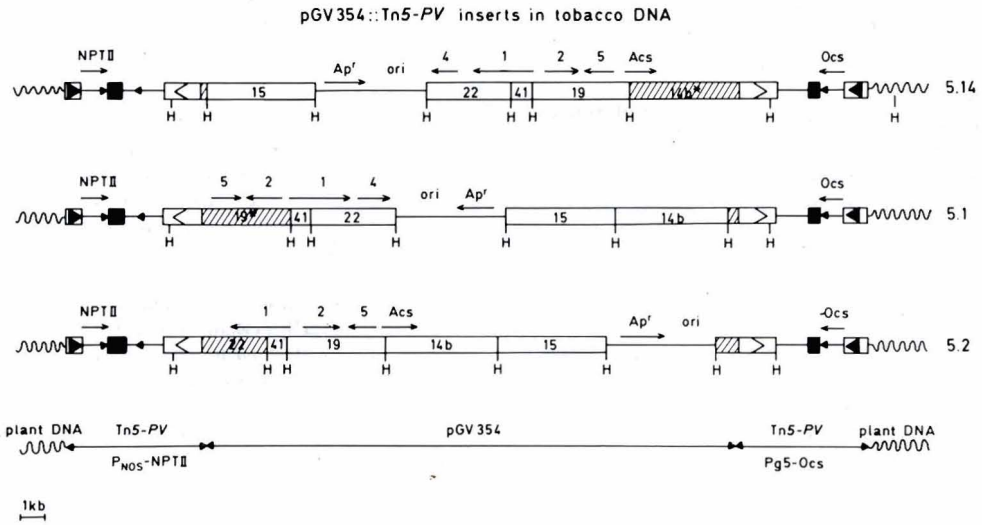


**Fig. 5.** Physical map of tobacco DNA containing pGV354::Tn*5-PV* derivatives 5.14, 5.1 and 5.2. H, *Hin*dIII; *Arrows* above the maps indicate the position of T-DNA encoded transcripts identified by RNA gel hybridization. *Ap$^r$* and *ori* show the position and polarity of pBR322 sequence. Other abbreviations are as in Fig. 1

in Fig. 4a). In DNA from 5.14 tissues only a single *Hin*dIII fragment is visible in the hybridization pattern obtained with probe *Hin*dIII (14b) because Tn*5-PV* was integrated close to the end of this fragment. The appearance of additional faint bands in the hybridization patterns obtained with probe *Hin*dIII (19) can be explained by the homology of gene 5 sequences, present in fragment *Hin*dIII (19), to the promoter region of gene 5 used as an element of the chimeric octopine synthase gene in Tn*5-PV*. The copy number of T-DNAs present in different tumour lines was

determined by using NPTII and *ocs* sequences, representing the left and the right ends of the T-DNA segment (Koncz and Schell 1986) as probes. Two copies of T-DNA insertions were detected in tumour tissues 5.12, 5.14 and 5.18, three copies in 5.1, 5.8 and four copies in 5.2 (data not shown). Due to the low copy number of T-DNA insertions in these different tumours any rearrangements in the structure of T-DNAs before or after integration would be visible by Southern gel hybridization experiments. Fragments lacking Tn*5-PV* insertions showed no alteration, while the size of junction fragments linking Tn*5-PV* to pGV354 sequences was identical in the plant DNA and in the corresponding pGV354::Tn*5-PV* plasmids. Southern hybridization data summarized in Fig. 4a therefore indicated that plasmid pGV354 and linked Tn*5-PV* sequences were transferred from *Agrobacterium* and maintained in plant cells apparently without any rearrangements. The structure of pGV354::Tn*5-PV* derivatives 5.2, 5.1 and 5.14 integrated in tobacco tumour DNAs is shown in Figure 5.

Further evidence to support these results was derived from hybridization analysis of T-DNA encoded transcripts in different tumours induced by pGV354::Tn*5-PV* derivatives. Poly(A)$^+$ RNA samples were purified from tumour lines 5.14, 5.1 and 5.12 and hybridized to the same probes as used in the DNA hybridization analysis. Figure 4b shows the absence of agrocinopine synthase transcripts (*Acs*) from 5.12 and 5.1 tumour tissues carrying a Tn*5-PV* insert in the *acs* gene, and the presence of transcripts 2, 4 and 5 in all three tumour lines. No additional transcripts hybridizing to pGV354 fragments were detected which is in accordance with previous results (Willmitzer et al. 1983).

## Discussion

A special plant gene vector has been designed which functions as a transposon in *E. coli* and as a plant gene vector when present in the appropriate *Agrobacterium* strain. The basis of this plant gene vector is transposon Tn*5* carrying a plant gene vector cassette (Koncz and Schell 1986). The plant gene vector cassette contains the plant marker genes neomycin phosphotransferase and octopine synthase, and the 25 bp border sequences needed for T-DNA transfer in *Agrobacterium*. The cassette also contains $ori_V$ and $ori_T$ sequences from plasmid RK2 which allow any plasmid that carries the Tn*5-PV* transposon to be mobilized from an *E. coli* strain harbouring RK2 helper functions (e.g., strain SM10) and to replicate in *Agrobacterium* strains, such as GV3101 (pMP90RK), providing RK2 replication helper functions. In consequence any DNA sequence, cloned in any *E. coli* vector, that consists of a set of different genes the function of which has to be analysed in plants, can be randomly mutagenized in *E. coli* by Tn*5-PV* insertion and the mutagenized DNA segments can be transferred to plant cells for functional analysis after mobilization to *Agrobacterium* GV3101 (pMP90RK). The physical mapping of the Tn*5-PV* inserts can also be readily performed after isolation of the mutagenized DNA from *E. coli* cultures.

To demonstrate the general applicability of this approach, a well-known DNA segment, the T-DNA of Ti plasmid pTiC58, was subjected to analysis by Tn*5-PV* insertion. Part of the T-DNA of pTiC58 was cloned in *E. coli* in pGV354 (Depicker et al. 1980). The function and structure of the tumour-inducing genes carried by this DNA segment are well known (Willmitzer et al. 1983; Joos et al. 1983). With this model system it was demonstrated that Tn*5-PV* could both mutagenize and elicit the *Agrobacterium*-mediated transfer of the mutagenized T-DNA segment in tobacco and kalanchoe plants.

It has been shown that the structure of the pGV354::Tn*5-PV* derivatives present in *E. coli* and the structure of the transferred DNA in tobacco cells were essentially identical, as expected for a transfer and integration mechanism involving the 25 bp T-DNA border sequences carried by Tn*5-PV*. These results therefore demonstrated that no major rearrangement of the cloned DNA had occurred on the way from *E. coli* via *Agrobacterium* to the plant nucleus.

There was also a strict correlation between the precise site of Tn*5-PV* insertion in pGV354 and the expected organogenic properties of the transformed plant cells. Thus insertions outside the known tumour-inducing genes 1, 2 and 4, did not result in any abnormal phenotype of the resulting tumours, whereas insertions in gene 1 (*IaaM*) led to teratoma formation and insertions in gene 4 (*ipt*) led to rooting tumours. Inserts in the *Acs* gene resulted in formation of tumours in which no agrocinopine (Ellis and Murphy 1981) was detected. These results, supported by data of transcript analysis, demonstrate that Tn*5-PV* provides a direct and simple method for the transfer, transcriptional mapping and functional analysis of large cloned DNA segments in plants.

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