MOLECULAR PLANT-MICROBE INTERACTIONS
Vol. 4, No. 2, pp. 155-162, 1991

The TR-DNA Region Carrying the Auxin Synthesis Genes of the *Agrobacterium rhizogenes* Agropine-Type Plasmid pRiA4: Nucleotide Sequence Analysis and Introduction into Tobacco Plants

Christine Camilleri and Lise Jouanin

Laboratoire de Biologie Cellulaire, Institut National de la Recherche Agronomique, 78026 Versailles Cedex, France.
Received 3 August 1990. Accepted 3 December 1990.

We have determined the nucleotide sequence of a 6-kilobase fragment of the *Agrobacterium rhizogenes* plasmid pRiA4 TR-region that carries genes (*aux1* and *aux2*) responsible for auxin biosynthesis in transformed plant cells. Sequence analysis revealed two open reading frames corresponding to proteins of 749 amino acids for the *aux1* gene and 466 amino acids for the *aux2* gene. We observed significant similarity between the amino acid sequences deduced from the pRiA4 *aux* genes and those of the auxin biosynthesis genes of *A. tumefaciens* octopine-type Ti plasmids, the *iaaM* and *iaaH* genes of *Pseudomonas savastanoi*, and different genes of the pRiA4 TL-region; however, the 5′-flanking regions of the pRi and pTi auxin biosynthesis genes were found to be completely different. Transgenic tobacco plants containing this entire 6-kilobase fragment of the pRiA4 TR-region have been obtained. Regenerated plants are phenotypically normal. The *aux1* gene is not or is very weakly expressed in these plants, but expression of the *aux2* gene leads to a modified root phenotype when plants are grown on medium containing an auxin precursor (naphthalene acetamide).

Crown gall and hairy root diseases induced by *Agrobacterium tumefaciens* (Smith and Townsend) Conn or *A. rhizogenes* (Riker *et al.*) Conn on dicotyledonous plants are the result of the transformation of plant cells by a specific DNA fragment, the T-DNA. This T-DNA, bordered by direct imperfect repeats of 24 base pairs (bp) required for its transfer, is part of a large bacterial plasmid, the tumor-inducing (Ti) or the root-inducing (Ri) plasmid (for reviews, see Melchers and Hooykaas 1987; Binns and Thomashow 1988). Crown gall and hairy root cells are able to proliferate in the absence of phytohormones, due to the expression of T-DNA genes, especially of genes involved in the synthesis of growth factors. In crown gall cells, the *tmr* gene is associated with cytokinin synthesis, and two genes, *tms1* and *tms2*, are involved in auxin production: the *tms1* gene product catalyzes the conversion of tryptophan to indole-3-acetamide (IAM), which is then converted to indole-3-acetic acid (IAA) by the product of the *tms2* gene. This biosynthetic pathway is not used in normal plant metabolism but occurs in the bacterium *Pseudomonas savastanoi* E. F. Smith, which harbors genes (*iaaM* and *iaaH*) similar to the *A. tumefaciens* auxin synthesis genes (Yamada *et al.* 1985). In agropine-type Ri plasmids, such as pRiA4 which we have studied, the T-region is split in two parts, TL and TR, and it has been shown by cross-hybridizations that the TR exhibits homology with pTi T-DNA only in loci responsible for agropine synthesis and in the *tms* locus (Willmitzer *et al.* 1982; Jouanin 1984; Huffman *et al.* 1984). The occurrence of two auxin synthesis genes has been shown by complementation of the pTi *tms* genes (White *et al.* 1985; Offringa *et al.* 1986). The right part of the pRiA4 TR-region harbors agropine synthesis genes, and the left part harbors the auxin synthesis genes. Here we present the 5,995 nucleotide sequence of the left part of the TR-region that carries the two auxin synthesis genes. The exact location of these genes has been determined, their coding sequences were compared to those of the homologous genes of *A. tumefaciens* and *P. savastanoi*, and their nontranslated regions were analyzed. (To avoid confusion, we chose here to systematically call the auxin synthesis genes *tms* when they are from *A. tumefaciens* and *aux* when they are from *A. rhizogenes*; the corresponding *P. savastanoi* genes will be named *iaaM* and *iaaH*.) The sequenced fragment was then introduced into tobacco and its effects in transgenic plants were studied.

Nucleotide and/or amino acid sequence data is to be submitted to GenBank, EMBL, and DDBJ as accession number JO3688.

MATERIALS AND METHODS

Plasmids, bacterial strains, and culture conditions. The cosmid pLJ85 containing the pRiA4 TR-region (Jouanin 1984) served as a source for the 6-kilobase (kb) *Sal*I fragment encompassing the left part of the TR-DNA. This 6-kb *Sal*I fragment was cloned in both orientations in pEMBL19 (Dente *et al.* 1985), giving plasmids named p19S7 and p19S25. To introduce the auxin synthesis genes in plants, the 6-kb *Sal*I fragment was cloned in the *Xba*I site of the binary vector pMRK62 (Vilaine *et al.* 1987), which confers kanamycin resistance to transformed plant cells; the resulting plasmid was designated pMRKS6 (Vilaine *et al.* 1987).

Strain HB101 of *Escherichia coli* (Boyer and Roulland-Dussoix 1969) was used for recombinant DNA techniques, and strain NM522 (Gough and Murray 1983) was used for the production of single-stranded DNA. They were grown at 37° C in Luria-Bertani medium. The filamentous phage M13K07 (Vieira and Messing 1987) was used for

the production of single-stranded DNA from pEMBL vectors for sequencing.

A. tumefaciens GV3101 (pMP90) (Koncz and Schell 1986), a rifampicin- and gentamycin-resistant derivative of nopaline *A. tumefaciens* C58, was grown at 28° C in Luria-Bertani medium supplemented with the appropriate antibiotics.

Antibiotic concentrations added in the bacterial media were 200 mg/L ampicillin, 10 mg/L tetracycline, and 50 mg/L kanamycin for *E. coli*, and 100 mg/L neomycin, 50 mg/L rifampicin, and 25 mg/L gentamycin for *A. tumefaciens*.

Recombinant DNA techniques for clone preparation. Most DNA techniques were performed essentially as described by Maniatis *et al.* (1982). Subclones presenting nested deletions of the cloned fragments were obtained from the plasmids p19S7 and p19S25 by random DNase I treatment according to Lin *et al.* (1985). Resulting subclones were sized on agarose gels before sequencing.

DNA sequencing and sequence analysis. DNA sequencing was conducted by the chain termination method of Sanger *et al.* (1981) on single-stranded DNA obtained from such subclones by using the phage M13K07 (Vieira and Messing 1987). When necessary, gaps were sequenced with synthetic oligonucleotide primers. Sequences were analyzed using the programs supplied by the computer services of CITI2 (BISANCE, French Ministry of Research and Technology, Paris).

Transformation of tobacco plants. *A. tumefaciens* GV3101 (pMP90) (Koncz and Schell 1986) was transformed by pMRKS6 according to Holsters *et al.* (1978). The resulting strain was used for *Nicotiana tabacum* L. (cv. Xanthi) leaf disk transformation as described by Budar *et al.* (1986). Kanamycin-resistant shoots were selected on Murashige and Skoog (1962) (MS) agar medium containing 100 mg/L of kanamycin and 500 mg/L of cefotaxime, then grown on MS medium without cefotaxime. Transformed plants were regenerated and analyzed by Southern and northern hybridizations using the 6-kb *Sal*I fragment as a probe as described by Jouanin *et al.* (1987).

RESULTS

We determined the complete nucleotide sequence of the pRiA4 6-kb *Sal*I fragment that covers the left part of the TR-region. A map of the sequenced region is shown in Figure 1, and the sequence is displayed in Figure 2. Jouanin *et al.* (1989) have established that the pRiA4 TR-region is flanked by 24-bp functional borders showing strong homology with the border consensus sequence of Ti plasmid T-DNA. We determined the left border sequence to be located at position 1,021 to 1,045 (Fig. 2). Eight open reading frames (ORFs) larger than 225 bp were found in the sequenced region (Fig. 1). Characteristics of these ORFs are given in Table 1. We have identified the two larger ORFs (6 and 2′) as the coding sequences of the pRiA4 auxin synthesis genes (*aux1* and *aux2*, respectively) by comparison with the auxin synthesis region of pTiAch5 (Gielen *et al.* 1984). As in the pTi genes, intervening sequences appear to be absent in both the *aux1* and *aux2* genes (Fig. 2).

Analysis of the *aux1* and *aux2* coding regions. The coding regions of the *aux* genes present significant sequence similarity with those of the pTiAch5 *tms1* and *tms2* genes (Gielen *et al.* 1984) and with the *iaaM* and *iaaH* genes of *P. savastanoi* (Yamada *et al.* 1985). The *aux1* gene was also compared with members of a pRiA4 TL-DNA gene family that present homologies with *tms1*: ORFs 8, 11 (*rolB*), 12 (*rolC*), 13, and 14 (Levesque *et al.* 1988). The coding region of the pRiA4 *aux1* gene (Fig. 3A) has an organization similar to ORF 8 (28% amino acid identity) and *tms1* (60% identity): in its N-terminal domain, the *aux1* gene product shows weak similarity (about 20%) with parts of the pRi ORFs 11, 12, 13, and 14, and the C-terminal region presents 52% identity with *iaaM* of *P. savastanoi*. The FAD-binding site of *tms1* (Klee *et al.* 1984), strongly conserved in *iaaM* (Yamada *et al.* 1985), is also present in ORF 8 and in the *aux1* gene product (Fig. 3A). The larger size of ORF 8 and the *aux1* and *tms1* genes compared to *iaaM* is due to the additional N-terminal polypeptide that is weakly homologous to the pRi TL-DNA ORFs 11, 12, 13, and 14, and whose function is unknown.

The *aux2* and *tms2* gene products can be aligned over the entire sequence with 71% homology, and there is 30% identity between *aux2* and *iaaH* (Fig. 3B) (27% for *tms2* and *iaaH*, Yamada *et al.* 1985).

Analysis of nontranslated regions of the *aux1* and *aux2* genes. The sequences of the regions comprised between the two *aux* genes and between the two *tms* genes (393 bp for pRiA4 and 345 bp for pTiAch5 [Gielen *et al.* 1984]), which contain possible promoter elements, were found to be largely different. The only homology is with a 12-bp sequence, located 76–87 bp upstream from the *aux2* ATG (position 2,912–2,923, Fig. 2) and 35–46 bp upstream from the *tms2* ATG.

The majority of the genes expressed in plants contain a TATA box (Joshi 1987; see Bruce and Gurley 1987; De Pater *et al.* 1987a; Bandyopadhyay *et al.* 1989 for T-DNA genes). TATA elements close to Joshi's consensus are found in the 5′ regions of the *aux1* gene (84 bp upstream from the ATG, position 3,134–3,146) and the *aux2* gene (79 bp from the ATG, position 2,927–2,915) (Fig. 2). For the *aux2* gene, the TATA motif is located in the 12-bp sequence

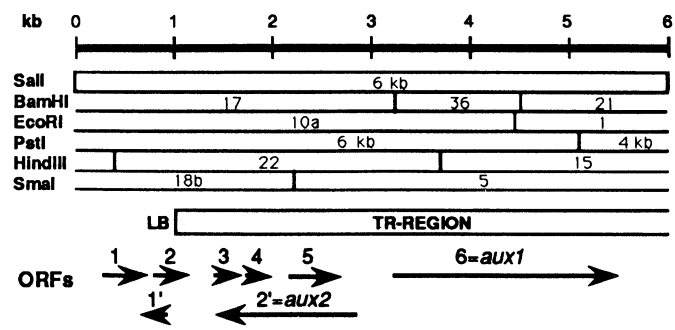

Fig. 1. Physical map of the sequenced region of the agropine-type plasmid pRiA4. Restriction fragment numbering or lengths are from Huffman *et al.* (1984) for *Hin*dIII, Jouanin (1984) for *Eco*RI and *Sma*I, Jouanin *et al.* (1986) for *Bam*HI, and L. Jouanin (unpublished) for *Pst*I and *Sal*I. The extent of the TR-region, delimited by the left border sequence (LB), is indicated by an open box. Arrows indicate position and polarity of the open reading frames (ORFs, see Table 1).

homologous to a sequence of the pTiAch5 and pTiA6NC *tms2* promoter, which contains a TATA element (Klee *et al.* 1984).

Sequences homologous to the polyadenylation signal AATAAA were found in the 3′ regions of the *aux1* and *aux2* genes (Fig. 2).

Many T-DNA genes contain upstream elements that modulate the transcriptional level (e.g. Ellis *et al.* 1987; Bouchez *et al.* 1989; Bruce *et al.* 1988; Leisner and Gelvin 1988, 1989; Mitra and An 1989). Sequences related to the 9-bp motif (TTTCAAGGA) observed by Lichtenstein *et al.* (1984) in a number of pTi T-DNA genes and by Klee *et al.* (1984) (TgTCAAcGA) 85 bp upstream from the pTiA6NC *tms1* translation start were found in the pRiA4 sequence: gTTCAAtGA at 349 bp upstream from the *aux1* ATG (position 2,881) and TTgaAAGGA at 385 bp from the *aux2* initiation codon (position 3,221). A sequence (CCAtATTGCCATG) 288 bp upstream from the pRiA4 *aux2* ATG (position 3,124) is related to a consensus sequence [CCACAN(T/A)NNNN(T/A)G] established by Memelink *et al.* (1987) from a transcriptional activator element found in the 5′-untranslated region of the pTi

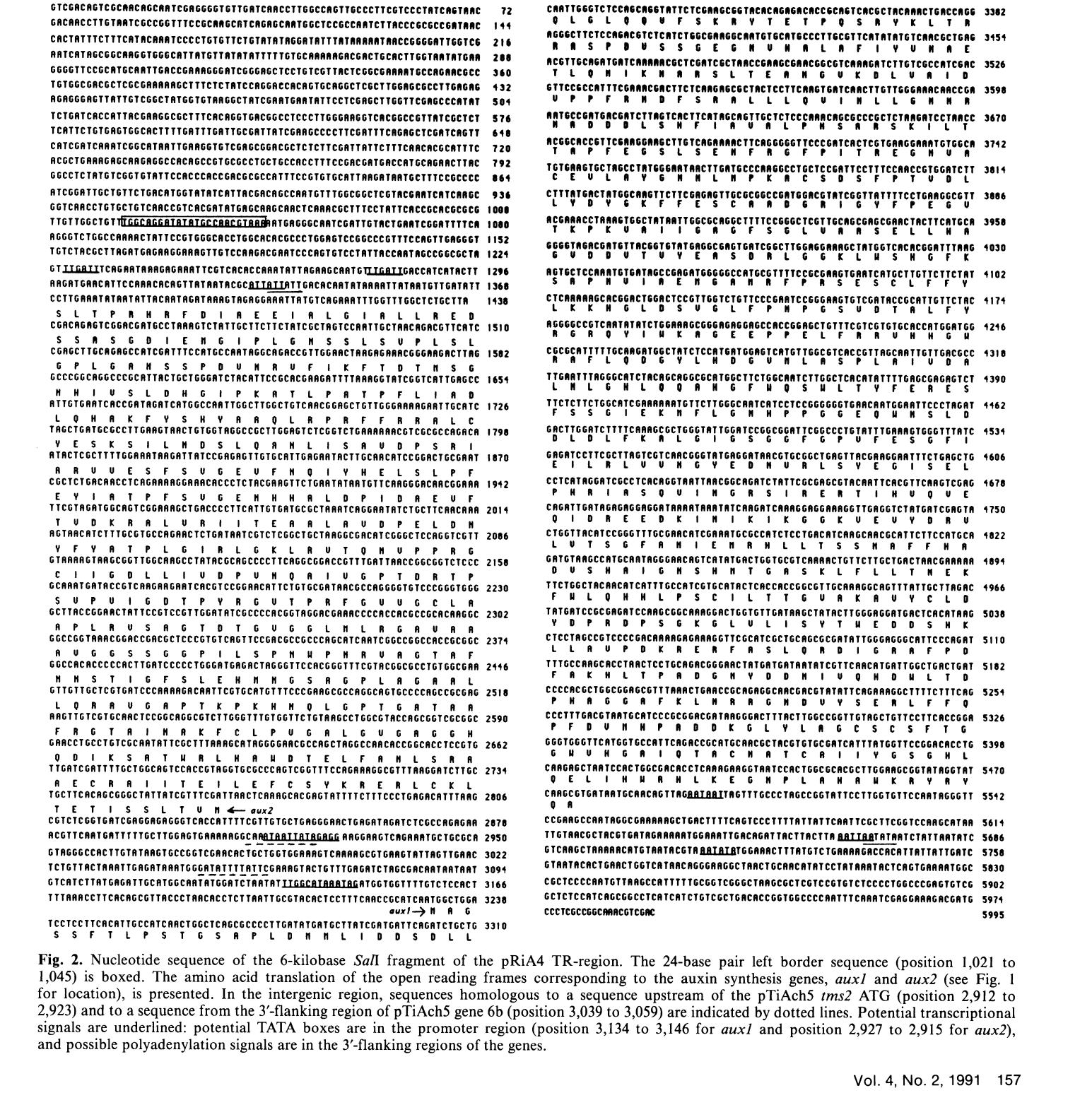

Fig. 2. Nucleotide sequence of the 6-kilobase *Sal*I fragment of the pRiA4 TR-region. The 24-base pair left border sequence (position 1,021 to 1,045) is boxed. The amino acid translation of the open reading frames corresponding to the auxin synthesis genes, *aux1* and *aux2* (see Fig. 1 for location), is presented. In the intergenic region, sequences homologous to a sequence upstream of the pTiAch5 *tms2* ATG (position 2,912 to 2,923) and to a sequence from the 3′-flanking region of pTiAch5 gene 6b (position 3,039 to 3,059) are indicated by dotted lines. Potential transcriptional signals are underlined: potential TATA boxes are in the promoter region (position 3,134 to 3,146 for *aux1* and position 2,927 to 2,915 for *aux2*), and possible polyadenylation signals are in the 3′-flanking regions of the genes.

tmr gene (De Pater *et al.* 1987b). A 21-bp motif (position 3,039–3,059, Fig. 2) is homologous (18 identical bases) to a sequence of the 3′-flanking region of pTiAch5 gene 6b (position 10,139–10,159; Gielen *et al.* 1984). Gene 6b is an oncogene (Hooykaas *et al.* 1988) that interacts with the auxin synthesis genes (Tinland *et al.* 1989). A search for a consensus sequence (GATAAATGNNATATTTNATTC) derived from these two 21-bp elements in the bank of nucleic acid sequences of GenBank has not allowed us to find this exact motif in other sequences.

Effects of the *aux* genes in transgenic tobacco plants. The entire 6-kb *Sal*I fragment carrying the two pRiA4 auxin synthesis genes was introduced into tobacco plants. We have obtained eight plants transformed by the two *A. rhizogenes aux* genes. Southern hybridization analysis of two of these plants, *Sal*6.1 and *Sal*6.5, which correspond to different transformation events, is presented in Figure 4. All the regenerated plants transformed by the two *A. rhizogenes aux* genes are fertile and show a normal phenotype. We were not able to detect transcripts corresponding to the *aux1* or *aux2* genes by northern hybridization of mRNA extracted either from the leaves or from the roots of transformed plants (data not shown).

To investigate the expression of the *aux* genes at the seedling stage, seeds of homozygous transformed plants *Sal*6.1 and *Sal*6.5 were germinated on MS medium supplemented with or without 1 mg/L of naphthalene acetamide (NAM, a more stable IAM analogue). Nontransformed seedlings develop normally on both media. On the medium without NAM, seeds of the *Sal*6.1 plant germinate normally, but seedlings of the *Sal*6.5 plant show longer and thicker roots with abundant root hairs (Fig. 5A). This modified root phenotype is the same as observed when normal seeds are germinated on medium containing low levels (0.1 mg/L) of naphthalene acetic acid (NAA). On the MS medium containing NAM, all the transformed seeds form modified roots and callus (Fig. 5B) as found when normal seeds are germinated on medium containing 1 mg/L of NAA. It can be seen that the response to NAM of *Sal*6.5 seedlings is more accentuated than that of *Sal*6.1 seedlings.

Cuttings of the *Sal*6.5 plant also showed modified roots on MS medium without NAM, whereas cuttings of untransformed and *Sal*6.1 plants root normally. Cuttings of *Sal*6.1 and *Sal*6.5 plants produce an abnormal root system on MS medium with 1 mg/L of NAM, while the untransformed plants develop a normal root system, and roots of *Sal*6.5 show a more marked phenotype than *Sal*6.1 (results not shown).

DISCUSSION

In this TR-DNA region, only two transcripts of 1.6 and 2.5 kb were observed by Taylor *et al.* (1985) in *N. glauca* Graham tumor lines transformed by pRiA4. The respective locations and sizes determined for the *aux2* (1,398 bp) and *aux1* (2,247 bp) ORFs are in agreement with these results. A single transcript of 1.6 kb was also observed in the region carrying the *aux2* ORF in transformed cucumber roots (J. Amselem, unpublished results). ORFs 3, 4, and 5 are located on the direct strand of the sequence in the region carrying on the complementary strand the *aux2* gene (Fig. 1). No transcript corresponding to these ORFs has been observed in this region, so there is no evidence that they correspond to functional genes in plants.

None of the ORFs located outside of the TR-region (1, 2, and 1′) showed a procaryotic ribosome binding site, and we do not know if they are actually transcribed in *Agrobacterium*. ORF 1 (147 amino acids) shows similarity with a 153-amino acid protein of the *E. coli* replication origin *oriC* (Buhk and Messer 1983) whose function is not known.

In plants, translation generally begins at the first AUG codon present on the mRNA, which is often flanked by the consensus sequence TAAACAATGGCT (Joshi 1987). For the *aux2* gene, a second ATG in frame is located at a distance of 354 bp downstream of the first one. Considering that the sequence flanking the first ATG (**AAAATGG**) matches Joshi's consensus, we suggest that it is the functional initiator of translation in plants. For the *aux1* gene, after the first ATG, we found two ATG codons in frame at the beginning of the ORF, 51 and 54 bp farther. The sequences flanking these three ATG codons are, respectively, GC**ATCAATGGCT**, CTT**GATATG**ATG, and GA**TATGATGCTT**. The first ATG environment corresponds well to Joshi's consensus, and there is no evidence that this ATG is not functional, but the exact determination of the initiation codon would need amino acid sequence determination of the translation product.

The coding regions of the pTiAch5 *tms* genes and the pRiA4 *aux* genes present the same organization and comparable sizes: 749 amino acids for *aux1* and 755 for *A. tumefaciens tms1*, 466 amino acids for *aux2* and 467 for *tms2*. The protein sequences deduced from the *A. rhizogenes* auxin synthesis genes are very similar to those of the corresponding genes of *A. tumefaciens*. This is consistent with the similarity of function of these proteins and an evolutionary relationship among these genes. The similarities observed between *aux1* and ORF 8 or *aux1* and *iaaM* are comparable to those reported for *tms1* and ORF 8 (31%, Levesque *et al.* 1988) and *tms1* and *iaaM* (50%, Yamada *et al.* 1985). Considering there is 38% homology between ORF 8 and *iaaM* (Levesque *et al.* 1988), *tms1* and ORF 8 are the most divergent, with *iaaM* closer to *tms1* than to ORF 8. The results obtained for the *aux1*

Table 1. Coordinates of open reading frames (ORFs) of more than 75 amino acids

ORF	First base	Last base	Number of amino acids	Calculated mol. wt.
Orientation a[a]				
1	284	724	147	16,072
2	800	1,162	121	13,055
3	1,410	1,658	83	8,662
4	1,733	1,987	85	9,038
5	2,163	2,663	167	18,055
6[b]	3,230	5,476	749	83,084
Orientation b[c]				
1′	885	658	76	8,789
2′[d]	2,836	1,439	466	49,477

[a] Orientation as read from left to right.
[b] ORF 6 corresponds to the *aux1* gene.
[c] Orientation as read from right to left.
[d] ORF 2′ corresponds to the *aux2* gene.

gene seem to indicate identical ways of evolution for *aux1* and *tms1*. ORF 8 is only weakly related to the *aux1* gene; an explanation could be that these sequences are derived from a common ancestor and that ORF 8 has evolved more rapidly than the others, perhaps due to the presence of the homologous *aux1* gene on the same plasmid. Whereas *aux2* and *tms2* have diverged from the *P. savastanoi iaaH* gene more than *aux1* and *tms1* from *iaaM*, they have only

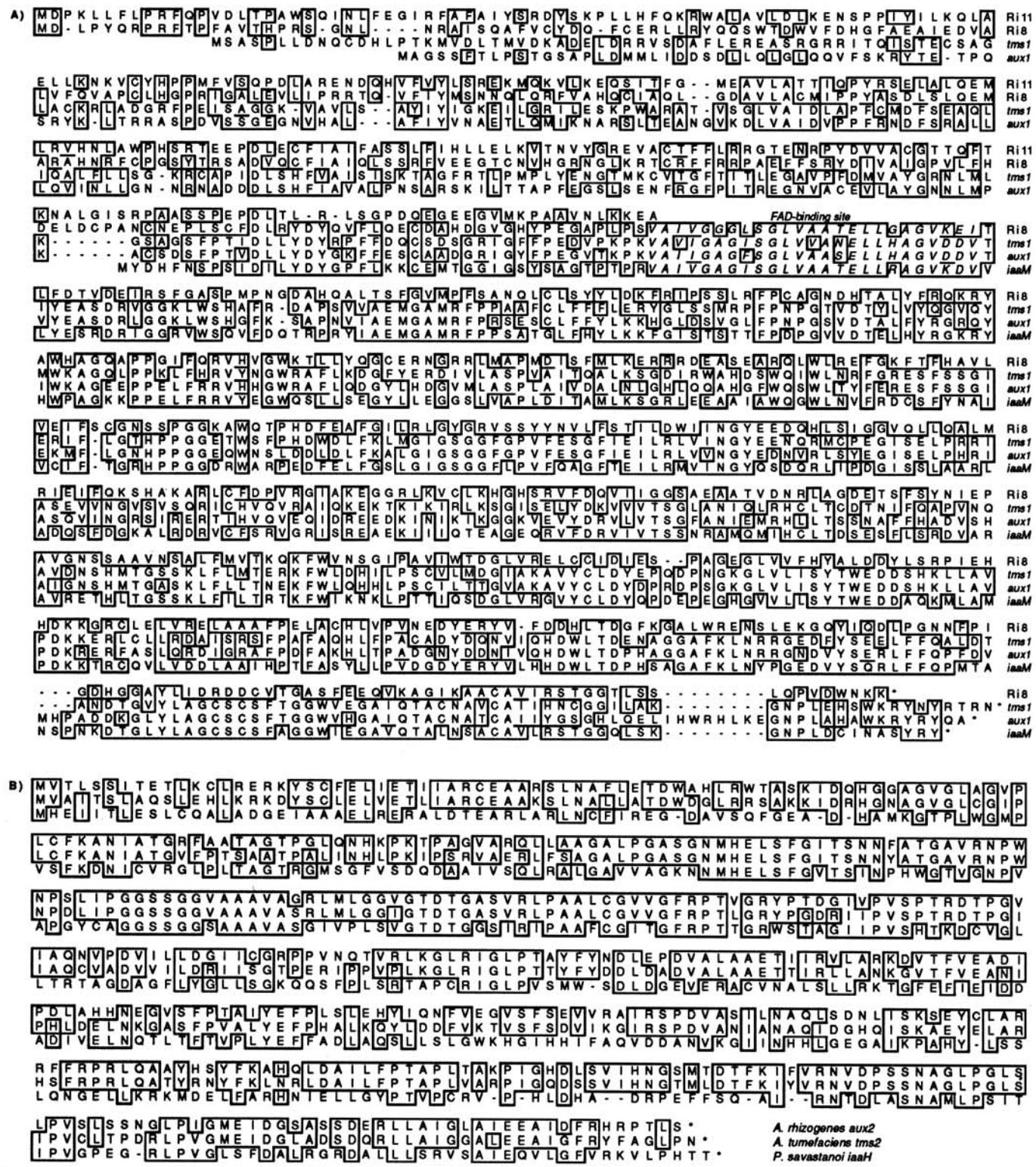

Fig. 3. Alignment of the protein sequences derived from the pRiA4 *aux* genes with those of related genes from pTiAch5 (Gielen *et al.* 1984), pRiA4 TL-DNA (Slightom *et al.* 1986), and *Pseudomonas savastanoi* (Yamada *et al.* 1985). Identical amino acids are boxed. **A**, Comparison of the pRiA4 *aux1* sequence with pTiAch5 *tms1*, pRiA4 TL-DNA ORFs 8 (Ri8) and 11 (Ri11), and *P. savastanoi iaaM*. The conserved FAD-binding site is in italics. **B**, Comparison of the pRiA4 *aux2* sequence with pTiAch5 *tms2* and *P. savastanoi iaaH* sequences.

slightly diverged from one another.

In contrast, the promoter regions of the auxin synthesis genes were found to be substantially different in Ti and Ri plasmids, which could be related to different forms of regulation. In fact, several loci in the pRi T-DNA are implicated in the control of root proliferation, which results from the influence of several different mechanisms (Cardarelli *et al.* 1985; Vilaine and Casse-Delbart 1987; Cardarelli *et al.* 1987). Auxin biosynthesis is only one of these mechanisms; other mechanisms are under the control of TL-DNA genes. It is possible to imagine in *A. rhizogenes* a particular set of mechanisms resulting in the determination of the hormonal balance in which the promoter activity level of the auxin synthesis genes could be regulated by other T-DNA gene products. Functional analysis of the promoter region will determine if the potential regulatory sequences found in this region are actually implicated in the transcriptional regulation of the pRiA4 auxin synthesis genes. The expression of the *aux1* and *aux2* genes with deleted promoters is being studied.

There is no report in the literature of plants containing both the *A. tumefaciens tms1* and *tms2* genes. Tobacco plants have been transformed by the *A. tumefaciens tms1* gene with its own promoter, giving phenotypically normal plants (Follin *et al.* 1985), whereas the *tms1* gene under the control of the cauliflower mosaic virus 19S promoter gave petunia and tobacco plants an extremely abnormal morphology due to high levels of IAM and IAA (Klee *et al.* 1987). High level of expression of the *tms1* gene alone is thus sufficient to lead to auxin levels resulting in highly modified plants. The plants transformed with the two pRiA4 auxin synthesis genes are phenotypically normal, indicating that the *aux1* gene is not sufficiently expressed to lead to auxin overproduction resulting in morphological alterations. The *aux1* gene appears therefore to be weakly active in seedlings and cuttings of certain *Sal*6 plants (i.e. *Sal*6.5), producing enough IAM and IAA to give slightly modified roots but not enough to deeply change the plant morphology. This modified root phenotype is soon outgrown to give normal plants, which may be related to the regulation of the genes in entire plants. Seeds and cuttings of plants transformed with the *aux2* gene are able to convert NAM into NAA, producing high levels of auxin that inhibit rooting, as has been found in transgenic plants expressing the *A. tumefaciens tms2* gene (Budar *et al.* 1986). Different levels of expression of the *aux* genes, leading to a more or less accentuated phenotype, have been observed. Preliminary results indicate that the level of DNA methylation of the *Sal*6.1 and *Sal*6.5 plants is not responsible for the differential levels of expression of the *aux* genes in these plants. The *aux2* gene under the control of its own promoter can be used by now in

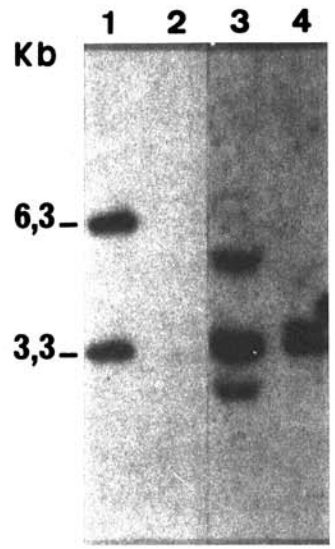

Fig. 4. Southern hybridization of *Hin*dIII-digested DNA with the 6-kilobase (kb) *Sal*I fragment. Lane 1 contains DNA from the plasmid pEMBL19 containing the 6-kb *Sal*I fragment. The 3.3-kb fragment is an internal fragment corresponding to *Hin*dIII fragment 22 in Figure 1. Lane 2 contains genomic DNA from a normal tobacco plant. Lanes 3 and 4 contain genomic DNA from tobacco plants containing the 6-kb *Sal*I fragment (lane 3, *Sal*6.1; lane 4, *Sal*6.5).

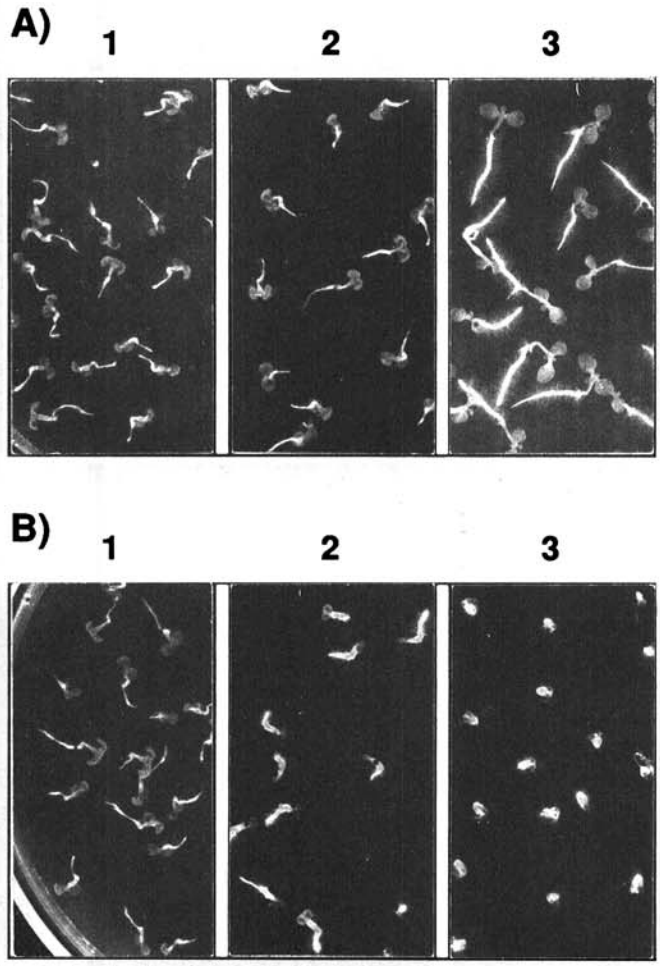

Fig. 5. Germination of seeds of wild-type and of homozygous transformed tobacco plants. **A**, Germination on Murashige and Skoog (1962) (MS) medium without naphthalene acetamide (NAM) (section 1, wild type; section 2, *Sal*6.1; and section 3, *Sal*6.5). Untransformed and *Sal*6.1 seeds germinate normally, but *Sal*6.5 seedlings show modified roots that are likely due to auxin overproduction. **B**, Germination on MS medium containing 1 mg/L of NAM (section 1, wild type; section 2, *Sal*6.1; and section 3, *Sal*6.5). Whereas the untransformed seeds germinate normally, seedlings expressing the *aux2* gene are able to convert NAM into active auxin and present a modified phenotype (shorter and thicker roots in the *Sal*6.1 seedlings) amplified in *Sal*6.5 (callus instead of roots).

negative selection.

Knowledge of the auxin biosynthesis genes can be a very useful tool in studying the processes of plant differentiation. The possibility to overproduce hormones in plants constitutes an important step in studying the expression of genes regulated by auxin level and determining the role of phytohormones and the way in which they act.

ACKNOWLEDGMENTS

We thank Pierre Rouzé and Mark Tepfer for critical reading of the manuscript.

LITERATURE CITED

Bandyopadhyay, R. S., Bruce, W. B., and Gurley, W. B. 1989. Regulatory elements within the agropine synthase promoter of T-DNA. J. Biol. Chem. 264:19399-19406.

Binns, A. N., and Thomashow, M. F. 1988. Cell biology of *Agrobacterium* infection and transformation of plants. Annu. Rev. Microbiol. 42:575-606.

Bouchez, D., Tokuhisa, J. G., Llewellyn, D. J., Dennis, E. S., and Ellis, J. G. 1989. The ocs-element is a component of the promoters of several T-DNA and plant viral genes. EMBO J. 8:4197-4204.

Boyer, H. W., and Roulland-Dussoix, D. 1969. A complementation analysis of the restriction and modification of DNA in *Escherichia coli*. J. Mol. Biol. 41:459-472.

Bruce, W. B., and Gurley, W. B. 1987. Functional domains of a T-DNA promoter active in crown gall tumours. Mol. Cell. Biol. 7:59-67.

Bruce, W. B., Bandyopadhyay, R., and Gurley, W. B. 1988. An enhancer-like element present in the promoter of a T-DNA gene from the Ti plasmid of *Agrobacterium tumefaciens*. Proc. Natl. Acad. Sci. USA 85:4310-4314.

Budar, F., Deboeck, F., Van Montagu, M., and Hernalsteens, J. P. 1986. Introduction and expression of the octopine T-DNA oncogenes in tobacco plants and their progeny. Plant Sci. 46:195-206.

Buhk, H. J., and Messer, W. 1983. The replication origin region of *Escherichia coli*: Nucleotide sequence and functional units. Gene 24:265-279.

Cardarelli, M., Spano, L., De Paolis, A., Mauro, M. L., Vitali, G., and Costantino, P. 1985. Identification of the genetic locus responsible for non-polar root induction by *Agrobacterium rhizogenes* 1855. Plant Mol. Biol. 5:385-391.

Cardarelli, M., Spano, L., Mariotti, D., Mauro, M. L., Van Sluys, M. A., and Costantino, P. 1987. The role of auxin in hairy root induction. Mol. Gen. Genet. 208:457-463.

Dente, L., Sallazo, L., Baldari, C., Cesareni, G., and Cortese, M. 1985. The pEMBL family of single-stranded vectors. Pages 101-107 in: DNA Cloning. D. M. Glover, ed. IRL Press, Washington, D.C.

de Pater, B. S., de Kam, R. J., Hoge, J. H. C., and Schilperoort, R. A. 1987a. Effects of mutations in the TATA box region of the *Agrobacterium* T-cyt gene on its transcription in plant tissues. Nucleic Acids Res. 15:8283-8292.

de Pater, B. S., Klinkhamer, M. P., Amesz, P. A., de Kam, R. J., Memelink, J., Hoge, J. H. C., and Schilperoort, R. A. 1987b. Plant expression signals of the *Agrobacterium* T-cyt gene. Nucleic Acids Res. 15:8267-8281.

Ellis, J. G., Llewellyn, D. J., Walker, J. C., Dennis, E. S., and Peacock, W. J. 1987. The *ocs* element: A 16 base pair palindrome essential for activity of the octopine synthase enhancer. EMBO J. 6:3203-3208.

Follin, A., Inzé, D., Budar, F., Genetello, C., Van Montagu, M., and Schell, J. 1985. Genetic evidence that the tryptophan 2-mono-oxygenase gene of *Pseudomonas savastanoi* is functionally equivalent to one of the T-DNA genes involved in plant tumour formation by *Agrobacterium tumefaciens*. Mol. Gen. Genet. 201:178-185.

Gielen, J., De Beuckeleer, M., Seurinck, J., de Greve, H., Lemmers, M., Van Montagu, M., and Schell, J. 1984. The complete nucleotide sequence of the TL-DNA of the *Agrobacterium tumefaciens* plasmid pTiAch5. EMBO J. 3:835-846.

Gough, G., and Murray, N. 1983. Sequence diversity among related genes for recognition of specific targets in DNA molecules. J. Mol. Biol. 166:1-19.

Holsters, M., de Waele, D., Depicker, A., Messens, E., Van Montagu, M., and Schell, J. 1978. Transfection and transformation of *Agrobacterium tumefaciens*. Mol. Gen. Genet. 163:181-187.

Hooykaas, P. J. J., den Dulk-Ras, H., and Schilperoort, R. A. 1988. The *Agrobacterium tumefaciens* T-DNA gene 6b is an *onc* gene. Plant Mol. Biol. 11:791-794.

Huffman, G. A., White, F. F., Gordon, M. P., and Nester, G. W. 1984. Hairy root inducing plasmid: Physical map and homology to tumor inducing plasmids. J. Bacteriol. 157:269-276.

Joshi, C. P. 1987. An inspection of the domain between putative TATA box and translation start site in 79 plant genes. Nucleic Acids Res. 15:6643-6653.

Jouanin, L. 1984. Restriction map of an agropine-type Ri plasmid and its homologies with Ti plasmids. Plasmid 12:91-102.

Jouanin, L., Tourneur, J., Tourneur, C., and Casse-Delbart, F. 1986. Restriction maps and homologies of the three plasmids of *Agrobacterium rhizogenes* strain A4. Plasmid 16:124-134.

Jouanin, L., Vilaine, F., Tourneur, J., Tourneur, C., Pautot, V., Muller, J. F., and Caboche, M. 1987. Transfer of a 4.3 kb fragment of the TL-DNA of *Agrobacterium rhizogenes* strain A4 confers the pRi transformed phenotype to regenerated tobacco plants. Plant Sci. 53:53-63.

Jouanin, L., Bouchez, D., Drong, R. F., Tepfer, D., and Slightom, J. L. 1989. Analysis of TR-DNA/plant junctions in the genome of a *Convolvulus arvensis* clone transformed by *Agrobacterium rhizogenes* strain A4. Plant Mol. Biol. 12:75-85.

Klee, H., Montoya, A., Horodyski, F., Lichtenstein, C., Garfinkel, D., Fuller, S., Flores, C., Peschon, J., Nester, E., and Gordon, M. 1984. Nucleotide sequence of the *tms* genes of the pTiA6NC octopine Ti plasmid: Two gene products involved in plant tumorigenesis. Proc. Natl. Acad. Sci. USA 81:1728-1732.

Klee, H., Horsch, R., Hinchee, M., Hein, M., and Hoffmann, N. 1987. The effects of overproduction of two *Agrobacterium tumefaciens* T-DNA auxin biosynthetic gene products in transgenic petunia plants. Genes Dev. 1:86-96.

Koncz, C., and Schell, J. 1986. The promoter of TL-DNA gene 5 controls the tissue specific expression of chimaeric genes carried by a novel type of *Agrobacterium* binary vector. Mol. Gen. Genet. 204:383-396.

Leisner, S. M., and Gelvin, S. B. 1988. Structure of the octopine synthase upstream activator sequence. Proc. Natl. Acad. Sci. USA 85:2553-2557.

Leisner, S. M., and Gelvin, S. B. 1989. Multiple domains exist within the upstream activator sequence of the octopine synthase gene. Plant Cell 1:925-936.

Levesque, H., Delepelaire, P., Rouzé, P., Slightom, J., and Tepfer, D. 1988. Common evolutionary origin of the central portions of the Ri TL-DNA of *Agrobacterium rhizogenes* and the Ti T-DNAs of *Agrobacterium tumefaciens*. Plant Mol. Biol. 11:731-744.

Lichtenstein, C., Klee, H., Montoya, A., Garfinkel, D., Fuller, S., Flores, C., Nester, E., and Gordon, M. 1984. Nucleotide sequence and transcript mapping of the *tmr* gene of the pTiA6NC octopine Ti-plasmid: A bacterial gene involved in plant tumorigenesis. J. Mol. Appl. Genet. 2:354-362.

Lin, H. C., Lei, S. P., and Wilcox, G. 1985. An improved DNA sequencing strategy. Anal. Biochem. 147:114-119.

Maniatis, T., Fritsch, E. F., and Sambrook, J. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 545 pp.

Melchers, L. S., and Hooykaas, P. J. J. 1987. Virulence of *Agrobacterium*. Oxford Surv. Plant Mol. Cell Biol. 4:167-220.

Memelink, J., de Pater, B. S., Hoge, J. H. C., and Schilperoort, R. A. 1987. T-DNA hormone biosynthetic genes: Phytohormones and gene expression in plants. Dev. Genet. 8:321-337.

Mitra, A., and An, G. 1989. Three distinct regulatory elements comprise the upstream promoter region of the nopaline synthase gene. Mol. Gen. Genet. 215:294-299.

Murashige, T., and Skoog, F. 1962. A revised medium for rapid growth and bioassay with tobacco tissue culture. Physiol. Plant. 15:473-497.

Offringa, I. A., Melchers, L. S., Regensburg-Tuink, A. J. G., Costantino, P., Schilperoort, R. A., and Hooykaas, P. J. J. 1986. Complementation of *Agrobacterium tumefaciens* tumor-inducing *aux* mutants by genes from the TR-region of the Ri plasmid of *Agrobacterium rhizogenes*. Proc. Natl. Acad. Sci. USA 83:6935-6939.

Sanger, F., Coulson, A. R., Barrel, B. G., Smith, A. J. H., and Roe, B. W. 1981. Cloning in single-stranded bacteriophage as an aid to rapid DNA sequencing. J. Mol. Biol. 143:161-178.

Slightom, J. L., Durand-Tardif, M., Jouanin, L., and Tepfer, D. 1986.

Nucleotide sequence analysis of TL-DNA of *Agrobacterium rhizogenes* agropine type plasmid: Identification of open-reading frames. J. Biol. Chem. 261:108-121.

Taylor, B. H., White, F. F., Nester, E. W., and Gordon, M. P. 1985. Transcription of *Agrobacterium rhizogenes* A4 T-DNA. Mol. Gen. Genet. 201:546-553.

Tinland, B., Huss, B., Paulus, F., Bonnard, G., and Otten, L. 1989. *Agrobacterium tumefaciens* 6b genes are strain-specific and affect the activity of auxin as well as cytokinin genes. Mol. Gen. Genet. 219:217-224.

Vieira, J., and Messing, J. 1987. Production of single-stranded plasmid DNA. Methods Enzymol. 153:3-11.

Vilaine, F., and Casse-Delbart, F. 1987. Independent induction of transformed roots by the TL and TR regions of the Ri plasmid of agropine type *Agrobacterium rhizogenes*. Mol. Gen. Genet. 206:17-23.

Vilaine, F., Charbonnier, C., and Casse-Delbart, F. 1987. Further insight concerning the TL region of the Ri plasmid of *Agrobacterium rhizogenes* strain A4: Transfer of a 1.9 kb fragment is sufficient to induce transformed roots on tobacco leaf fragments. Mol. Gen. Genet. 210:111-115.

White, F. F., Taylor, B. H., Huffman, G. A., Gordon, M. P., and Nester, E. W. 1985. Molecular and genetic analysis of the transferred DNA regions of the root inducing plasmid of *Agrobacterium rhizogenes*. J. Bacteriol. 164:33-44.

Willmitzer, L., Sanchez-Serrano, J., Buschfeld, E., and Schell, J. 1982. DNA from *Agrobacterium rhizogenes* is transferred to and expressed in axenic hairy root plant tissues. Mol. Gen. Genet. 186:16-22.

Yamada, T., Palm, C. J., Brooks, B., and Kosuge, T. 1985. Nucleotide sequence of the *Pseudomonas savastanoi* indoleacetic acid genes show homology with *Agrobacterium tumefaciens* T-DNA. Proc. Natl. Acad. Sci. USA 82:6522-6526.