JOURNAL OF BACTERIOLOGY, Jan. 1994, p. 495–503
0021-9193/94/$04.00+0

Vol. 176, No. 2

Opine-Regulated Promoters and LysR-Type Regulators in the Nopaline (*noc*) and Octopine (*occ*) Catabolic Regions of Ti Plasmids of *Agrobacterium tumefaciens*

JOHANNES VON LINTIG, DIETER KREUSCH, AND JOACHIM SCHRÖDER*

Institut für Biologie II, Universität Freiburg, D-79104 Freiburg, Germany

Received 3 July 1993/Accepted 16 November 1993

Essential steps in the uptake and catabolism of the plant tumor metabolites nopaline and octopine in *Agrobacterium* spp. are performed by proteins encoded in the nopaline catabolic (*noc*) and octopine catabolic (*occ*) regions of Ti plasmids. We investigated the opine activation of the genes by using (i) promoter studies of *Agrobacterium* spp. and (ii) analysis of the promoter interaction with the regulatory proteins NocR (*noc*) and OccR (*occ*). The *noc* region contained two nopaline-induced promoters (Pi1[noc] and Pi2[noc]) and one autogenously regulated promoter (Pr [control of NocR expression]). Pi2 and Pr overlapped and were divergently oriented (Pi2/Pr[noc]). DNA binding studies and DNase I footprints indicated that NocR bound specifically to single binding sites in Pi1[noc] and Pi2/Pr[noc] and that Pi2 and Pr were regulated from the same binding site. The binding was independent of the inducer nopaline, and nopaline caused small changes in the footprint. The promoters in the *noc* and *occ* regions shared sequence motifs and contained the sequence T-N_{11}-A, which is characteristic for LysR-type-regulated promoters. The *occ* region contained one octopine-induced and one autogenously regulated promoter (Pi/Pr[occ]) in the same arrangement as Pi2/Pr[noc] in the *noc* region. Promoter deletions indicated that sequences flanking the OccR binding site determined the extent of induction, although they did not bind OccR. The promoter bound OccR in the absence and presence of octopine. The opine caused a change in the mobility of the DNA-protein complex with the complete promoter. The resected fragments did not reveal this opine-induced shift, and it was also not detectable with the DNA-NocR complexes with the two promoters of the *noc* region.

Agrobacterium tumefaciens induces tumors on a wide range of gymnosperms and dicotyledonous angiosperms by transfer of a part of the tumor-inducing (Ti) plasmid DNA into the plant cells (see references 13, 38, and 42 for recent reviews). Genes in the transferred DNA are responsible for the neoplastic properties of the transformed cells and for the synthesis of unusual substances (opines), which can serve as growth substrates for the bacteria. That benefit was the driving force in the evolution of this unusual plant-microbe interaction (11).

The utilization of the opines nopaline [N^2-(1,3-D-dicarboxypropyl)-L-arginine; nopaline-type Ti plasmids] and octopine [N^2-(1-D-carboxyethyl)-L-arginine; octopine-type Ti plasmids] has been well investigated (see reference 31 for a review). Ti plasmid functions are required in both cases, and Fig. 1 summarizes the genetic and functional organization of the catabolic regions. The nopaline catabolic (*noc*) region in pTiC58 is split into two parts separated by several kilobase pairs with unknown functions. The genes identified in the left part code for catabolic enzymes, and those in the right part encode four polypeptides involved in opine uptake. Expression of the genes is regulated by one nopaline-induced promoter in each region (Pi1[noc] and Pi2[noc]). Both are controlled by a positive regulator (NocR) encoded in the right part of the *noc* region (*nocR*). The octopine catabolic (*occ*) region contains a single octopine-induced promoter (Pi[occ]) controlling the expression of all catabolic functions (Fig. 1). The regulation is mediated by *occR*, which is transcribed divergently to Pi[occ].

Our previous results showed that the regulatory proteins NocR and OccR are related to each other (35) and to a family of other positive regulators (LysR type) (12). Here, we describe the identification of the promoter sequences and their interaction with the regulatory proteins. The results show that NocR binds to both promoters in the *noc* region in the absence and presence of nopaline and that the opine induces small changes in DNase I footprints. The binding sites share significant similarities to each other and to the previously identified (36) OccR binding site in Pi[occ]. The promoter assays in *Agrobacterium* strains indicated that sequences flanking the binding sites influenced the induction. This was investigated with Pi[occ] in the *occ* region by promoter deletion analysis and gel retardation analysis of the OccR-DNA interactions. The results indicated that the OccR binding site was necessary for induction but that the flanking sequences determined the extent of induction, although they revealed no binding of OccR.

MATERIALS AND METHODS

***Agrobacterium* strains and plasmids.** APF2 is a plasmid-free derivative of LBA275 (also known as C58C1) (15). pGV3850 (41) was derived from nopaline plasmid pTiC58 and contains the complete *noc* region. Plasmid pGV2260 (6), which contains the complete *occ* region, was a derivative from octopine-type plasmid pTiB6S3.

***Escherichia coli* strains and plasmids.** The pTZ18R/19R vector system (40), *E. coli* JM109 and JM105 (39), and the expression vectors pKK233-2 (2) and pINIIA2 (22) have been described previously. *E. coli* S17-1 was used for the conjugation of plasmids to the *Agrobacterium* strains; it contains the *trans*-acting mobilization factors of plasmid RP4 integrated in the chromosome (32). pFDY180, a vector containing multiple cloning sites, a promoterless *lacZ* reporter gene, and a univer-

* Corresponding author. Mailing address: Institut für Biologie II, Universität Freiburg, Schänzlestrasse 1, D-79104 Freiburg, Germany. Phone: 49-761 203 2691. Fax: 49-761 203 2601.

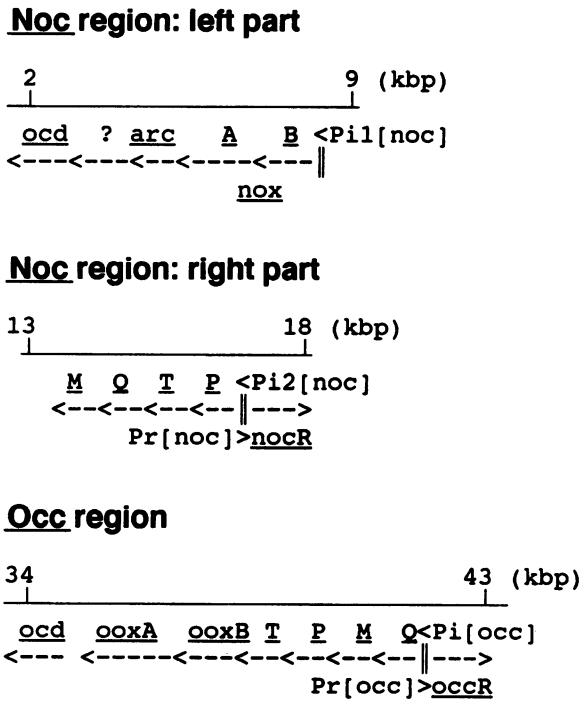
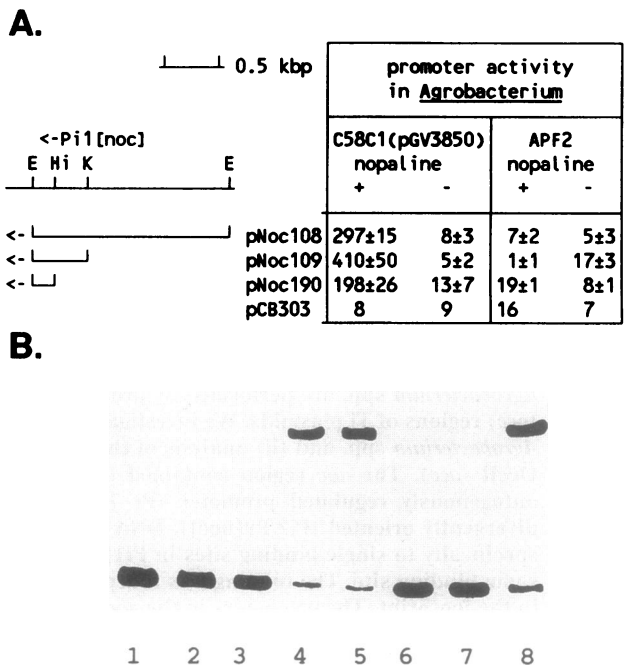


FIG. 1. Organization of the *noc* region in nopaline plasmid pTiC58 (two parts, separated by sequences with unknown functions) and the *occ* region of octopine plasmid pTiAch5. The map coordinates refer to an *Sma*I site conserved in the DNA regions transferred to the plant cells (7). Abbreviations: *ocd*, ornithine cyclodeaminase; ?, gene for a 40-kDa protein with an unknown function; *arc*, arginase; *noxA*/*noxB* and *ooxA*/*ooxB*, two proteins necessary for nopaline and octopine oxidase activities, respectively; *M*, *Q*, *P*, and *T*, coding regions for four proteins necessary for opine uptake; *nocR* and *occR*, regulatory proteins necessary for activation of the inducible promoters (<Pi) which control the expression of the catabolic functions; Pr>, promoter for expression of *occR* or *nocR*.



	promoter activity in Agrobacterium			
	C58C1(pGV3850) nopaline		APF2 nopaline	
	+	-	+	-
pNoc108	297±15	8±3	7±2	5±3
pNoc109	410±50	5±2	1±1	17±3
pNoc190	198±26	13±7	19±1	8±1
pCB303	8	9	16	7

FIG. 2. (A) Promoter region of Pi1[noc], subfragments cloned in broad-host-range vector pCB303, and activity in promoter test assays (β-galactosidase units ± standard error of the mean [see Materials and Methods]). Arrows with the subfragments indicate the position and orientation of the *lacZ* reporter gene. E, *Eco*RI; K, *Kpn*I; Hi, *Hin*fI. (B) Gel retardation assays with the 184-bp *Eco*RI-*Hin*fI promoter fragment and NocR expressed in *E. coli*. Lanes: 1, DNA fragment; 2, plus 2 μg of protein from NocR-free extract; 3, like lane 2, but plus 0.1 mM nopaline; 4, 2 μg of NocR extract (pNocEx expressed in *E. coli*); 5, like lane 4, but plus 0.1 mM nopaline; 6, like lane 4, but plus 200-fold excess of unlabelled *Eco*RI-*Hin*fI fragment (Pi1[noc]); 7, like lane 4, but plus a 200-fold excess of the unlabelled *Hin*dIII-*Nae*I fragment from the Pi2/Pr[noc] region (Fig. 3A); 8, like lane 4, but plus 0.8 mM nopaline.

sal primer site, was a gift from B. Rak (University of Freiburg, Freiburg, Germany).

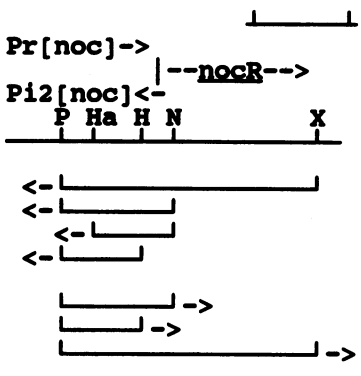
Growth media. The bacteria were grown in Luria-Bertani medium (1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl, 0.2% glycerol, 0.02% $MgSO_4$ [pH 7.5]) at 28°C (*A. tumefaciens*) or 37°C (*E. coli*) with the appropriate antibiotics (*A. tumefaciens*, 100 μg of rifampin per ml, 100 μg of carbenicillin per ml, 2 μg of tetracycline per ml; *E. coli*, 15 μg of tetracycline per ml, 50 μg of ampicillin per ml).

Promoter test plasmids for *Agrobacterium* strains. The constructs were based on pCB303, a broad-host-range vector containing a small polylinker and the promoterless *lacZ* reporter gene (28). The Ti plasmid fragments were inserted into the unique cloning sites (*Xba*I, *Pst*I, *Bam*HI), and suitable sites for unidirectional insertion were usually obtained via intermediate plasmids in the vectors pTZ18R/19R and pINIIA2. The plasmids were conjugated (1) from *E. coli* S17-1 to the *Agrobacterium* strains APF2, C58C1(pGV3850), and C58C1 (pGV2260). The presence of plasmids in *Agrobacterium* strains was routinely confirmed by miniscale isolation of the plasmids, retransformation of *E. coli* JM109, and restriction analysis.

Determination of promoter activities in *Agrobacterium* strains. Liquid cultures were grown overnight in Luria-Bertani medium at 28°C and harvested by centrifugation, and the cells were resuspended to an A_{600} of 0.4 in AB glucose medium (0.3% K_2HPO_4, 0.1% NaH_2PO_4, 0.1% NH_4Cl, 0.03% $MgSO_4$, 0.02% KCl, 0.001% $CaCl_2$, 0.0003% $FeSO_4$, 0.5% glucose [pH 7.0]) with (0.4 mM) or without the opines. The cultures were incubated for 6 h on a rotary shaker (200 rpm) at 28°C. They were harvested by centrifugation and resuspended to an A_{600} of 0.5 to 0.7 in buffer containing 60 mM Na_2HPO_4, 40 mM NaH_2PO_4, 10 mM KCl, 1 mM $MgSO_4$, and 50 mM 2-mercaptoethanol. The cells were broken by vortexing after addition of 30 μl of 0.05% sodium dodecyl sulfate (SDS) and 30 μl of chloroform. The β-galactosidase assays have been described previously (35). The enzyme units are defined as 1,000 × A_{410}/time (min) × A_{600} of the culture (28). The analysis was routinely performed with cultures from four independent colonies.

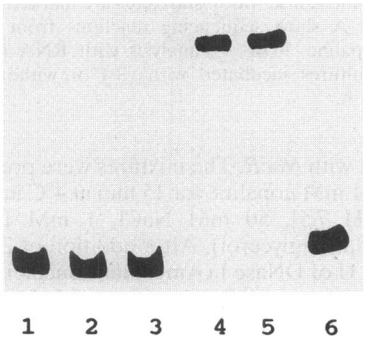
Primer extension analysis. The experiments for Pi1[noc] were performed with pNoc110 (35) maintained in *A. tumefaciens* C58C1(pGV3850). The plasmid pNoc110 contains a 3.2-kbp *Bam*HI-*Sma*I fragment from the upstream region of *noxB* cloned in pCB303. The assays for Pi2[noc] were carried out with pNoc105 (35) in *A. tumefaciens* APF2. The plasmid harbors a 1.6-kbp *Pst*I-*Xba*I fragment from the right end of the *noc* region cloned in pCB303; it contains *nocR* and 592 bp upstream of the start ATG. Duplicate cultures were grown overnight in Luria-Bertani medium, diluted 1:50 into the same medium, and allowed to grow to an A_{600} of 0.3. Nopaline (0.4 mM) was then added to one of the duplicates, and the cultures

A.



	promoter activity in Agrobacterium			
	C58C1(pGV3850) nopaline		APF2 nopaline	
	+	-	+	-
pNoc105	657±20	18±6	818±21	18±5
pNoc172	311±15	16±4	47±12	32±19
pNoc184	454±21	16±1	54±1	71±8
pNoc151	9±3	10±2	14±3	13±3
pNoc173	21±1	20±4	210±17	162±4
pNoc150	40±2	37±1	30±3	33±2
pNoc131	12±1	10±3	6±1	3±2

B.



C.

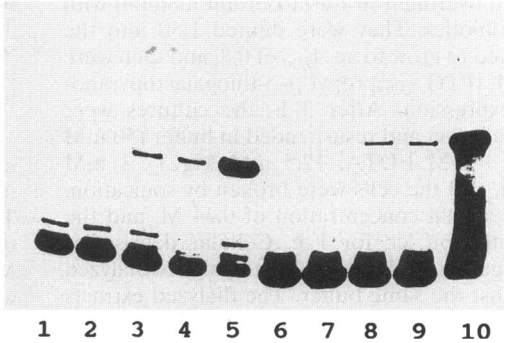


FIG. 3. (A) Promoter region of Pi2/Pr[noc], subfragments cloned in pCB303, and activity in promoter test assays (β-galactosidase units ± standard error of the mean [see Materials and Methods]). Arrows with the subfragments indicate the position and orientation of the *lacZ* reporter gene. Ha, *Hae*III; H, *Hin*dIII; N, *Nae*I; P, *Pst*I; X, *Xba*I. (B) Gel retardation assays with the 444-bp *Hae*III-*Nae*I fragment and NocR expressed in *E. coli*. Lanes: 1, DNA fragment; 2, plus control extract (NocR free [2 μg]); 3, like lane 2, but plus 0.1 mM nopaline; 4, NocR extract (2 μg); 5, like lane 4, but plus 0.1 mM nopaline; 6, like lane 4, but with 200-fold excess of unlabelled DNA fragment. (C) Comparison of gel retardation assays performed with extracts from *Agrobacterium* strains and *E. coli*. Lanes 1 to 5 were run with the 172-bp *Hin*dIII-*Nae*I fragment from Pi2Pr[noc] (Fig. 3A); lanes 6 to 10 were run with the 184-bp *Eco*RI-*Hin*fI fragment from Pi1[noc] (Fig. 2A). Lanes: 1 and 6, extract from NocR-free *Agrobacterium* strain (APF2 [8 μg of protein]); 2 and 7, like lane 1, but plus 0.1 mM nopaline; 3 and 8, extract from *Agrobacterium* strain expressing NocR (pNoc105 in APF2 [8 μg]); 4 and 9, like lane 3, but plus 0.1 mM nopaline; 5 and 10, extract with NocR expressed in *E. coli* (pNocEx [2 μg]).

were incubated for another 5 h. Total RNA was prepared according to the method of van der Meer et al. (34). The cells were harvested by centrifugation, resuspended in buffer (20 mM Na-acetate, 1 mM EDTA, 0.5% SDS [pH 5.5]), and extracted twice at 60°C with phenol saturated with 20 mM Na-acetate (pH 5.5). The nucleic acids were purified by three precipitations with ethanol. The oligonucleotide 5′-CGGCC AAGTTTTGAGGAGG-3′ was used to detect *noxB* transcripts (Pi1[noc]), and 5′-CGGGACAGCCGAAAATGAC GCC-3′ was used to identify *nocP* transcripts (Pi2[noc]). The primers were end labeled with [γ-^{32}P]ATP (110 TBq/mmol [Amersham Buchler, Braunschweig, Germany]) and annealed to approximately 50 μg of RNA. The rest of the procedure has been described previously (24). Sequence ladders from the same regions were obtained with the same oligonucleotides, and the subfragments were cloned in pTZ18R.

Resection of the Pi[occ] promoter with exonuclease III. The deletions were performed with a 433-bp *Hin*dIII-*Pst*I fragment containing the Pi/Pr[occ] region. Restriction sites suitable for the deletions were obtained via an intermediate clone in pTZ18R. The digestions were performed with the fragment cloned in vector pFDY180, and the restriction site configuration is shown in Fig. 7A (pOcc200), which also summarizes the results. The deletions were performed from both sides with the double-stranded Nested Deletion Kit (Pharmacia LKB Biotechnology) according to the manufacturer's recommendations. The mutants were transformed into *E. coli* JM109, and the extent of the deletions was determined by DNA sequence analysis. The fragments were recloned into pCB303 for the promoter assays of the *Agrobacterium* strains.

Plasmids for expression of OccR and NocR. The plasmids for expression of the regulators in *Agrobacterium* strains have been described before (pNoc105 for *nocR*, pOcc265 for *occR*) (35). The proteins were expressed in both cases from their own promoters. The expression in *E. coli* required additional cloning steps because it was desirable to express the proteins from their deduced start ATGs. The same strategy was used for expression of NocR and OccR. DNA fragments containing the coding regions were subcloned in vector pTZ19R, and the nucleotides next to the start ATGs were modified by site-directed mutagenesis to create an *Nco*I site. The mutagenesis was performed with single-stranded DNA produced with helper phage M13K07 in *E. coli* RZ1032 (16) as described before (19). *NocR* was mutagenized with the oligonucleotide 5′-GGAGAAAG**CCATGG**TTCAATCGCG-3′, and *occR* was mutagenized with 5′-GATGTTATGGTG**CCATGG**TGAAT

CTCAGGC-3′ (the created *Nco*I site is boldfaced and underlined). The mutations were verified with restriction digests and DNA sequence analysis. To eliminate the possibility of artifacts occurring during the mutagenesis, the nonsequenced regions were exchanged as far as possible against fragments from the parent plasmids with help of suitable restriction sites. The fragments were then recloned with *Nco*I and *Hin*dIII into expression vector pKK233-2. This fused the protein start ATG directly to the inducible *trc* promoter of the vector. The plasmids were named pNoxEx (NocR) and pOcc411 (OccR). Test experiments showed that overexpression of OccR with the pKK233-2-based plasmid was lethal to *E. coli*. It was therefore necessary to reclone the expression unit (vector promoter plus *occR* coding region) as a *Bam*HI-*Xba*I fragment into the low-copy-number vector pCB303. This plasmid was named pOcc410. The expression of the proteins was confirmed by labelling experiments with minicells (*E. coli* DS410) (26).

Extracts for DNA binding assays. The cultures with pNocEx (in *E. coli* JM105), pOcc410 (in *E. coli* JM109), or vector plasmids were grown overnight in Luria-Bertani medium with the appropriate antibiotics. They were diluted 1:50 into the same medium, allowed to grow to an A_{600} of 0.8, and then were induced with 1 mM IPTG (isopropyl-β-D-thiogalactopyranoside) for protein expression. After 3 h, the cultures were harvested by centrifugation and resuspended in buffer (50 mM Tris-HCl [pH 7.5], 1 mM EDTA, 12.5 mM $MgCl_2$, 1 mM β-mercaptoethanol), and the cells were broken by sonication. NaCl was added to a final concentration of 0.44 M, and the lysates were incubated on ice for 1 h. Cellular debris was removed by centrifugation, and the supernatants were dialyzed for at least 3 h against the same buffer. The dialyzed extracts were centrifuged, brought to a final glycerol concentration of 20%, and stored at −70°C. Protein concentrations were determined according to the method of Lowry et al. (21). The *Agrobacterium* experiments were performed with extracts from strain APF2 transformed with either pCB303 (control), pNoc105 (*nocR*), or pOcc265 (*occR*). The growth of the cultures and the extract preparation were as described for *E. coli*, except for the omission of IPTG and harvest of the cultures after they reached an A_{600} of 2 to 3.

Promoter fragments for gel retardation assays. The probe for Pi1[noc] was a 184-bp *Eco*RI-*Hin*fI fragment (Fig. 2), and for Pi2/Pr[noc], we used a 444-bp *Hae*III-*Nae*I fragment (restriction sites suitable for the labelling were obtained from subclones in the polylinker of vector pTZ19R). For the *occ* promoter region Pi/Pr[occ], we employed the *Eco*RI-*Hin*dIII fragment isolated from pOcc200 (see Fig. 7A) and various resected fragments from the exonuclease III deletions of pOcc200. The fragments were labelled by filling in with the Klenow subunit of DNA polymerase and [α-^{32}P]dATP (24). The DNA was precipitated with isopropanol, washed twice with 70% ethanol, and redissolved in Tris-EDTA buffer.

Gel retardation assays. Gel retardation assays were carried out according to published procedures (8). The incubations (final volume, 25 μl) contained buffer (10 mM Tris-HCl [pH 7.5], 50 mM NaCl, 3 mM $MgCl_2$, 1 mM EDTA, 10% glycerol), 1 μg of calf thymus DNA, approximately 10,000 cpm of labelled promoter fragment, and protein extract as indicated in the figure legends. The samples were analyzed after 10 to 15 min by electrophoresis in 5% polyacrylamide gels (buffer, 50 mM Tris-HCl [pH 8]–0.38 M glycine–1 mM EDTA; conditions, 4 h, 40 mA, 4°C).

DNase I footprint analysis. DNase I footprint analysis was performed with published techniques (9). The incubation mixtures contained approximately 100,000 cpm of end-labelled promoter fragment, 5 μg of calf thymus DNA, and 30 μg of crude extract with *NocR*. The mixtures were preincubated with or without 0.1 mM nopaline for 15 min at 4°C in buffer (10 mM Tris-HCl [pH 7.5], 50 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol, 5% glycerol). After addition of 2 μl of 250 mM $MgCl_2$ and 1 U of DNase I (Amersham Buchler), the digestion was allowed to proceed for 3 min at 4°C. It was stopped by addition of 4 μl of 0.5 M EDTA and heating for 10 min at 65°C. The mixtures were extracted twice with phenol-chloroform (50:50 [vol/vol]), and the DNA was precipitated with ethanol. The dried DNA was redissolved in formamide, and portions (20,000 cpm) were analyzed in a 5% denaturing long-range sequencing gel (U.S. Biochemical Corp.). The Maxam-Gilbert sequencing reactions were performed as described previously (30).

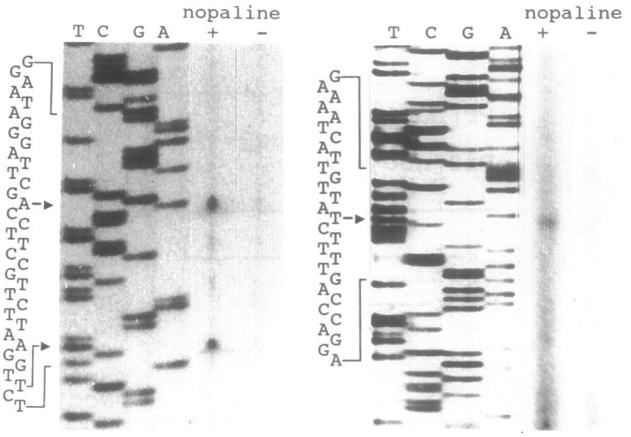


FIG. 4. Primer extension analysis of *noxB* (left, Pi1[noc]) and *nocP* (right, Pi2[noc]). The DNA sequences are shown in the 5′-to-3′ direction, and the transcription start sites are marked by arrows. Lanes T, C, G, and A show sequencing reactions from the same DNA sequence. Nopaline + or −, analysis with RNA (50 μg) from *A. tumefaciens* cultures incubated with (+) or without (−) 0.4 mM nopaline for 5 h.

Miscellaneous methods. DNA sequence analysis was performed with the dideoxy nucleotide chain termination technique as described previously (35, 43), either with single-stranded DNA (pTZ18R/19R clones) or with double-stranded DNA (pFDY180-based deletion clones) and the universal sequence primer site in pFDY180. The DNA polymerization reactions were carried out with [α-^{35}S]dATP (>37 TBq/mmol [Amersham Buchler]) and modified T7 DNA polymerase (Sequenase [U.S. Biochemical Corp.]). Standard molecular analysis techniques were performed according to published procedures (24).

RESULTS

Pi1[noc] and binding of NocR. The nopaline-induced promoter Pi1[noc] is located upstream of the catabolic genes in the left part of the *noc* region (Fig. 1), and its activation is strictly dependent on the presence of *nocR* (35). Assays with several fragments cloned into the promoter test vector pCB303 showed that a 184-bp *Eco*RI-*Hin*fI subfragment was sufficient for inducibility (Fig. 2A [pNoc190]). The data with larger fragments were variable, suggesting that the flanking sequences influenced the promoter activity (pNoc108, pNoc109). The 184-bp fragment was used for gel retardation assays with NocR expressed in *E. coli* and with control extracts (Fig. 2B). The results indicated that NocR bound specifically to the promoter, because a protein-DNA complex was observed only

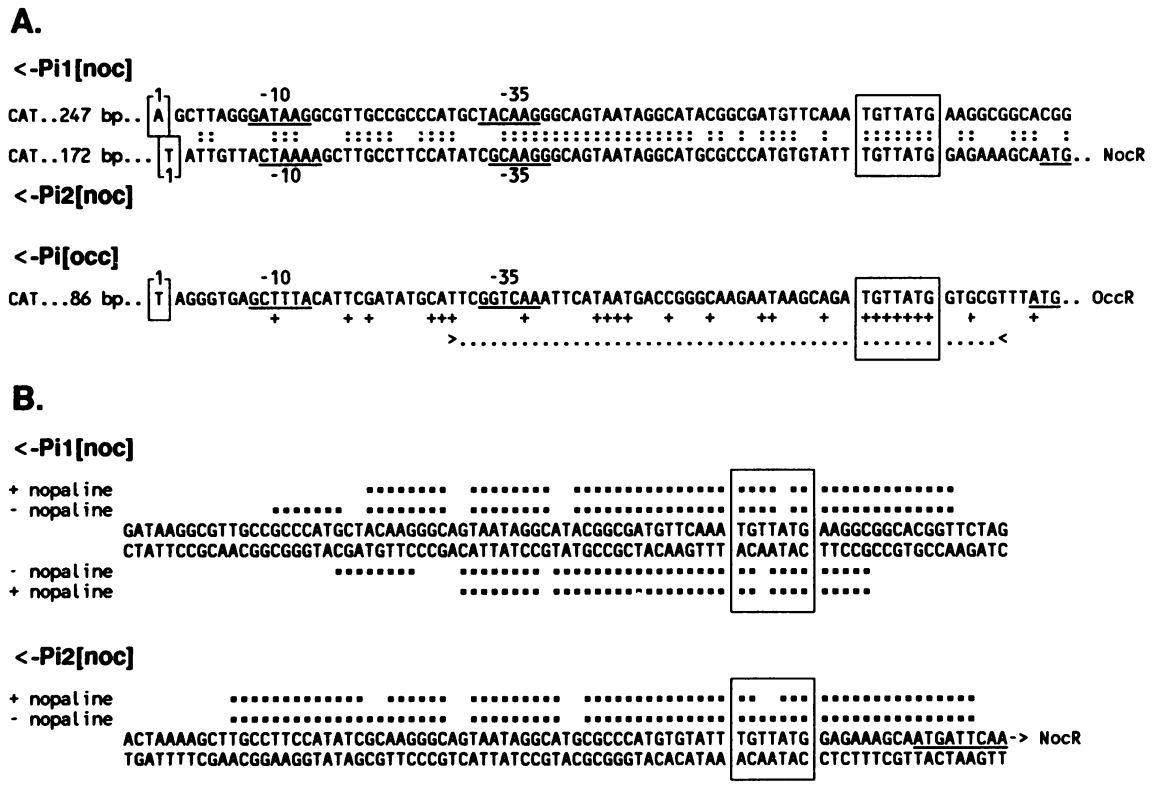


FIG. 5. (A) Alignment of the Pi1[noc], Pi2[noc], and Pi[occ] sequences containing the binding sites for the regulators NocR and OccR. The arrows indicate the direction of opine-inducible transcription. Transcription start sites are boxed (data for Pi[occ] are from Wang et al. [36]). The −10 and −35 regions of the inducible promoters and start ATGs of NocR and OccR (in the sequences of Pi2[noc] and Pi[occ], respectively) are underlined. Double dots indicate sequence identity. Plus signs below the Pi[occ] sequence indicate sequences identical in all three promoters; the 7-bp motif common to all three promoter regions is boxed. Small dots indicate the region protected in Pi/Pr[occ] by OccR (from Wang et al. [36]). (B) Regions protected (large dots) by NocR in the promoters Pi1[noc] and Pi2/Pr[noc] in the absence (−) and presence (+) of nopaline.

in the presence of NocR-containing extracts and because the complex formation was strongly reduced by an excess of unlabelled fragment. The binding was independent of nopaline, and there was no significant difference in the migration of the protein-DNA complex in the absence or presence of the opine (Fig. 2B, lanes 4 and 5). The same result was obtained with up to 0.8 mM nopaline. No complex was observed after addition of a *Hin*dIII-*Nae*I fragment containing Pi2[noc], the second inducible promoter from the right part of the *noc* region (Fig. 1), indicating that the two promoters competed for NocR.

Pi2/Pr[noc] and binding of NocR. We have previously demonstrated (35, 43) that a 1.6-kbp *Pst*I-*Xba*I fragment (pNoc105) from the right part of the *noc* region contains the regulatory gene *nocR* and two divergent promoters which control *nocR* (Pr[noc]) and the inducible expression of the genes for opine transport proteins (Pi2[noc]) (Fig. 1). Experiments with smaller fragments located Pi2[noc] in a 444-bp *Hae*III-*Nae*I fragment (Fig. 3A [pNoc184]). The additional removal of 172 bp at the right end of pNoc184 abolished the promoter activity completely (pNoc151), indicating that the sequences between the *Hin*dIII and *Nae*I sites were essential. This placed Pi2[noc] very close to the start of the *nocR* coding region.

The regulation of Pr[noc] was investigated with plasmids which contained fragments in the opposite orientation with respect to the *lacZ* reporter gene (Fig. 3A). Interestingly, pNoc173 revealed high levels of promoter activity in APF2 but not in C58C1(pGV3850). The only known difference between the two strains was Ti plasmid pGV3850, which expressed *nocR* from its *noc* region, and therefore this result indicated that NocR repressed its own expression. This conclusion was supported by pNoc131 (Pr[noc] plus complete *nocR*) because it had very low activity not only in C58C1(pGV3850) but also in APF2. The same type of regulation had been suggested for OccR (36). As with Pi2[noc], removal of the sequences between the *Hin*dIII and *Nae*I sites abolished the promoter activity of Pr[noc] (pNoc150). Taken together, the results indicated that essential elements of the two divergent promoters were located in the 172-bp *Hin*dIII-*Nae*I fragment which covered the sequences directly in front of the *nocR* coding region.

Gel retardation assays were performed with the 444-bp *Hae*III-*Nae*I fragment from pNoc184 and NocR expressed in *E. coli*. The results (Fig. 3B) showed the formation of a specific NocR-DNA complex in the absence and presence of nopaline, and, as with P1[noc], there was no significant difference in the migration of the protein-DNA complex in the absence or presence of the opine. The same results were obtained with the 172-bp *Hin*dIII-*Nae*I subfragment (data not shown).

The *noc* region promoters are not active or induced by nopaline in *E. coli* (data not shown), and therefore it was an interesting question whether *Agrobacterium* functions contributed to the protein-DNA complex. This was investigated with extracts from control APF2 and APF2(pNoc105), which expressed NocR from its own promoter in *Agrobacterium* strains. The labelled fragments were from Pi[noc] (184-bp *Eco*RI-*Hin*fI fragment, pNoc190 [Fig. 2a]) and from Pi2/Pr[noc] (172-bp *Hin*dIII-*Nae*I fragment [Fig. 3A]). The results in Fig.

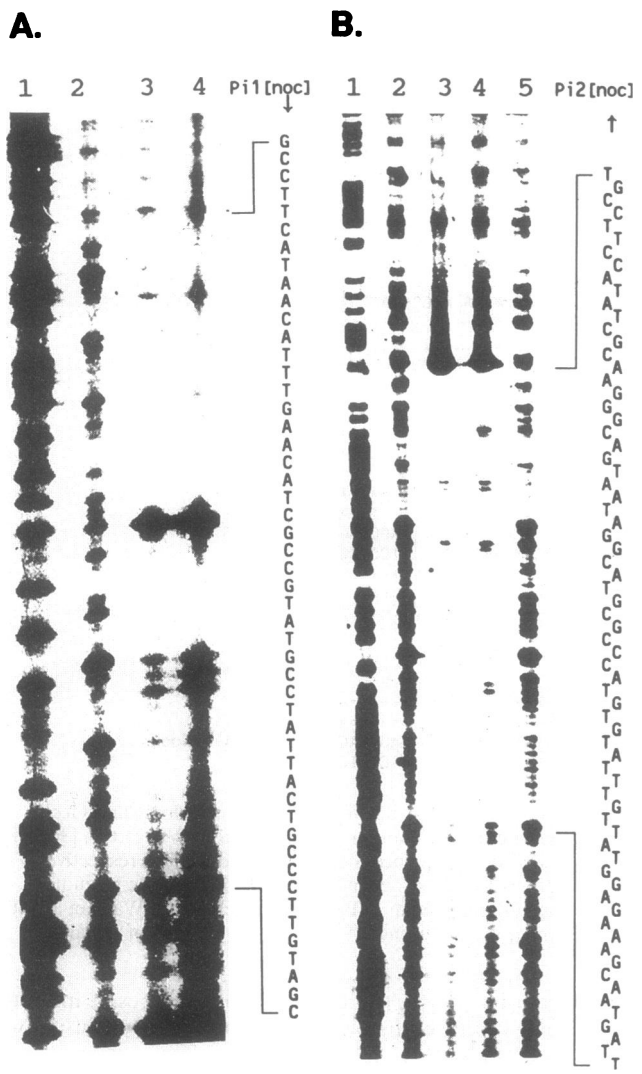


FIG. 6. DNase I footprint analysis of the nopaline-induced promoters of the *noc* region with NocR expressed in *E. coli*. Arrows indicate the direction of the inducible promoters. (A) Pi1[noc]. The reactions were performed with the 461-bp *Eco*RI-*Kpn*I fragment from pNoc109 (Fig. 2A). Lanes: 1, Maxam-Gilbert AG reaction; 2, DNase I (1 U); 3, DNase I plus NocR extract (30 μg); 4, like lane 3, but plus 0.1 mM nopaline. (B) Pi2[noc]. The reactions were performed with the 444-bp *Hae*III-*Nae*I fragment from pNoc184 (Fig. 3A). Lanes: 1, Maxam-Gilbert AG reaction; 2 and 5, DNase I (1 U); 3, DNase I plus NocR extract (30 μg); 4, like lane 3, but plus 0.1 mM nopaline.

3C showed that a protein-DNA complex was detectable only with extracts from APF2(pNoc105) and that the complexes migrated to the same positions, regardless of the NocR expression in *E. coli* or *Agrobacterium* strains. This suggested that other *Agrobacterium* factors did not significantly contribute to the DNA-protein complex. The experiments were also carried out with extracts from APF2(pNoc105), which had been incubated in vivo for 6 h with 0.4 mM nopaline. The NocR-DNA complexes migrated to the same position as before (data not shown), suggesting that NocR and its binding to the DNA were not modified in the presence of the opine to such an extent that differences were detectable in the assay system. The noninducibility of the *noc* region promoters in vivo in *E. coli* may indicate that NocR cannot activate transcription by the *E. coli* RNA polymerase. Alternatively, it cannot be excluded that the uptake of the inducer nopaline is so low that the intracellular concentration is not sufficient for promoter induction.

***noc* region: transcription start sites and DNase I footprints.** The primer extension analysis of the two nopaline-induced promoters is shown in Fig. 4 (left, Pi1[noc]; right, Pi2[noc]). In both cases, extension products were obtained only with RNA from *A. tumefaciens* induced with nopaline. The presence of two bands with Pi1[noc] suggested two potential transcription start sites, but the extensive overall similarity between the opine-induced promoters (discussed below [see Fig. 5A for a summary]) supported the notion that the shorter transcript represented a degradation product or a truncated version of the longer transcript.

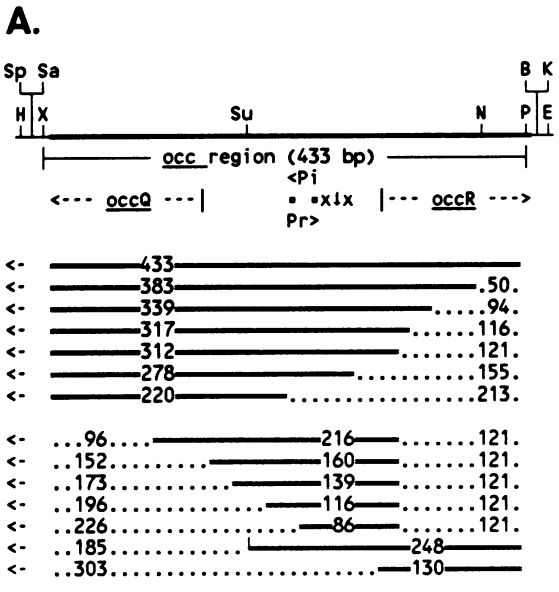
Comparison of the Pi1[noc] and Pi2/Pr[noc] promoter regions showed that the 70 bp preceding the start ATG of NocR had extensive similarities to sequences in the Pi1[noc] fragment (Fig. 5A). The similarity to Pi[occ] was less pronounced, but all three promoters shared the 7-bp motif TGTTATG, which is part of the region protected by OccR in Pi[occ] (36). The motif is unique in the *occ* and *noc* regions (total sequence, 19 kbp [references 25, 27, 29, 35 and 43 and unpublished data]).

The footprint analysis (Fig. 6 [see Fig. 5B for the detailed sequences]) showed that NocR bound to the conserved regions and that the protected sequences were slightly different in the absence and presence of nopaline. A comparison with OccR/Pi[occ] (36) indicated that NocR covered a larger sequence stretch than OccR. The binding sites for OccR/PiPr[occ] and NocR/Pi2Pr[noc] were very close to the start of the coding regions for the regulators, and the binding site of NocR included its own start ATG (Fig. 5B). Taken together, the results indicated that the induction of the nopaline-induced promoter Pi2[noc] and the repression of Pr[noc] by NocR were mediated from the same binding site.

Resection analysis of Pi[occ] and binding of OccR. The binding site of OccR to Pi/Pr[occ] had been defined previously (Fig. 5A [36]). Experiments in our laboratory indicated that the flanking sequences played an important role in the induction, and therefore we decided to analyze the region of the inducible promoter Pi[occ] in more detail. This was performed with exonuclease III deletions from both sides of the 433-bp *occ* region fragment that contained the OccR binding site and parts of the coding sequences for *occQ* and *occR* (Fig. 7A). The data with strain APF2 showed basic levels of promoter activity and no stimulation in the presence of octopine. This was expected, because *occR* was truncated in all cases and because APF2 has no functional *occR* equivalent encoded in the chromosome.

A 50-bp deletion upstream of Pi[occ] reduced the inducible promoter activity sixfold in strain C58C1(pGV2260) (pOcc300 versus pOcc301a [Fig. 7A]). This was unexpected, because the deleted sequences were within the *occR* coding region and were not part of the OccR binding site. More extensive deletions within the *occR* coding region had no additional drastic effects (pOcc332a, pOcc329a, pOcc303a). The deletion of further sequences (OccR binding) led to a complete loss of the inducible promoter activity (pOcc363a, pOcc317a). The results indicated that the OccR binding site was essential but that the 5′ end of the *occR* coding region was required for optimal induction of Pi[occ].

The deletions from the opposite side were performed with pOcc303a, which contained the shortest fragment with inducible promoter activity. The removal of 96 bp from the coding region for OccQ increased the activity sixfold (pOcc307), and the values were comparable to those of the complete promoter



	β-galactosidase activity (U) in Agrobacterium			
	C58C1(pGV2260) octopine		APF2 octopine	
pOcc200	+	-	+	-
pOcc300	749±43	34±4	15±8	11±3
pOcc301a	122± 9	10±5	9±2	7±1
pOcc332a	117± 1	21±8	18±6	14±3
pOcc329a	155± 5	18±6	11±5	11±5
pOcc303a	157± 4	19±7	13±6	12±4
pOcc363a	24± 6	21±8	17±3	16±8
pOcc317a	21± 8	16±8	15±2	14±4
pOcc307	745±31	29±14	49± 3	41± 6
pOcc315	303±43	31±16	36±10	29± 7
pOcc331	634±28	30± 2	38±23	49±10
pOcc328	686±63	31±17	33±14	26±12
pOcc327	16± 5	11± 3	60± 7	68± 5
pOcc302	318±25	14± 2	24± 5	30± 6
pOcc312	n.t.	n.t.	-	-

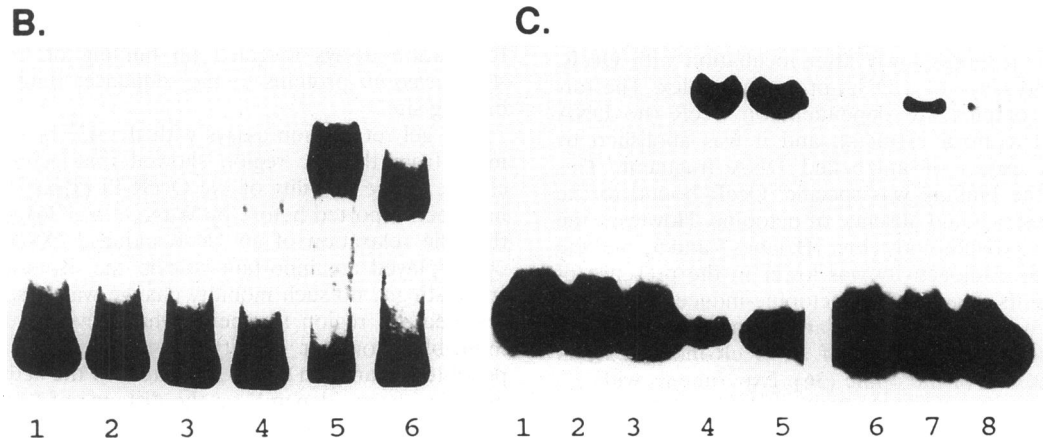


FIG. 7. (A) Pi/Pr[occ] promoter region, resected fragments, and promoter activities in *Agrobacterium* strains. (Top left) Restriction site configuration for the exonuclease III resections. B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; K, *Kpn*I; N, *Nae*I; P, *Pst*I; Sa, *Sal*I; Sp, *Sph*I; Su, *Sau*3A1; X, *Xba*I. The figure also shows the translation starts of *occQ* and *occR*, the −10 and −35 regions (▪) of Pi[occ] and Pr[occ], respectively, and the OccR binding region with the DNA bend center (x ↓ x) (36). (Bottom left) Position of the resected fragments and the *lacZ* reporter gene (←). Dots represent deleted *occ* region sequences, and the numbers indicate the lengths in base pairs. (Right) Promoter activities (± standard error of the mean) in plasmid-free APF2 and in C58C1(pGV2260) in the absence and presence of octopine. n.t. or -, not tested. (B) Gel retardation assays with OccR expressed in *E. coli* (pOcc410) and the labelled 500-bp *Eco*RI-*Hin*dIII fragment from pOcc300 (Fig. 7A). Lanes: 1, DNA fragment; 2, plus control extract from *E. coli* (OccR free [2 μg]); 3, like lane 2, but plus 0.1 mM octopine; 4, OccR extract (2 μg) plus 0.1 mM octopine plus 200-fold excess unlabelled promoter fragment; 5, OccR extract (2 μg) without octopine; 6, like lane 5, but plus 0.1 mM octopine. (C) Comparison of gel retardation assays performed with OccR expressed in *E. coli* (lanes 1 to 5) and *Agrobacterium* strains (lanes 6 to 8) and the 116-bp resected fragment from pOcc328 (Fig. 7A). Lanes: 1, DNA fragment; 2, plus *E. coli* control extract (2 μg); 3, like lane 2, but plus 0.1 mM octopine; 4, extract with OccR (2 μg); 5, like lane 4, but with 0.1 mM octopine. Lane 6, control *Agrobacterium* extract (APF2 [15 μg]); 7, OccR expressed in *Agrobacterium* strain from pOcc265 (15 μg); 8, like lane 7, but plus 0.1 mM octopine.

fragment in pOcc300 (Fig. 7A). Extending the deletions up to a total of 200 bp led to variable results (compare pOcc315, pOcc331, pOcc328), but all of the fragments revealed much higher values for induced activity than those of the starter fragment in pOcc303a. The differences showed no correlation with the length of the fragments, indicating that the high values were not simply a result of shortening the distance between the promoter and reporter genes. This notion is supported by the results with pOcc302. This plasmid contained the *Sau*3A1-*Pst*I fragment in the right half of the fragment in pOcc300 (Fig. 7A), with the left end in a position intermediate between the ends of pOcc331 and pOcc328, which both revealed very high levels of promoter activity. pOcc302, however, had only about 50% of that activity, although it also contained the upstream sequences which were required for optimal inducibility, as suggested by the data with the 5′-resected fragments. A further deletion of 30 bp abolished the activity completely (pOcc327), and inspection of the DNA sequences showed that they contained the −10 region of Pi[occ] (36).

Taken together, the results indicated that sequences to the right and the left of the OccR binding site strongly influenced the promoter activity, and therefore the binding of OccR was

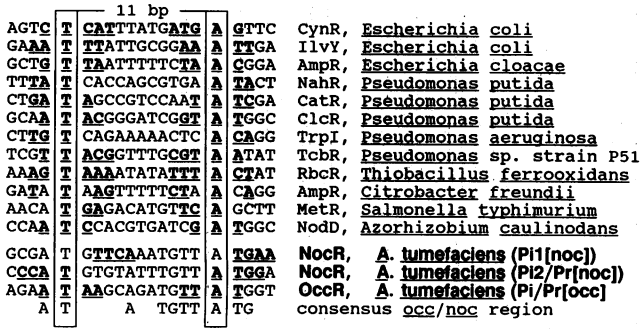


FIG. 8. Sequence comparison of promoters regulated by different LysR-type proteins. The invariant T-N_{11}-A motif is boxed. Short inverted repeats around the conserved T and A residues are underlined. Sequence sources: CynR, *E. coli* (33); IlvY, *E. coli* (37); AmpR, *Escherichia cloacae* and *Citrobacter freundii* (20); NahR, *Pseudomonas putida* (14); CatR, *P. putida* (23); ClcR, *P. putida* (5); TrpI, *Pseudomonas aeruginosa* (4); TcbR, *Pseudomonas* sp. strain P51 (34); RbcR, *Thiobacillus ferrooxidans* (17); MetR, *Salmonella typhimurium* (3); NodD, *Azorhizobium caulinodans* (10).

also investigated. The first experiments were performed with the 500-bp *Eco*RI-*Hin*dIII fragment from pOcc300 (complete 433-bp Pi/Pr[occ] promoter region) and OccR expressed from pOcc410 in *E. coli*. Figure 7B shows that the labelled DNA fragment migrated more slowly after incubation with OccR, indicating the presence of a DNA-protein complex. The formation of the complex was dependent on OccR (no DNA retardation with control extracts), and it was abolished by addition of an excess of unlabelled DNA fragment. This indicated that the binding was specific. OccR bound to the DNA in the presence and absence of octopine. However, the complexes migrated differently (Fig. 7B, lanes 5 and 6), and the amount of bound radioactivity was lower in the presence of octopine than in its absence. This octopine-induced migration change had been described before, and it had been proposed that it was caused by relaxation of an OccR-induced DNA bend in the presence of the opine (36). Experiments with the resected promoters (data not shown) revealed that OccR was bound by fragments containing the complete OccR binding site (pOcc303a, pOcc328, pOcc327 [see Fig. 7A for the positions of the fragments]), but there was no detectable DNA-OccR complex formation with fragments containing only parts of the binding site (pOcc363a) or the flanking sequences to the left (pOcc317a) or to the right (pOcc312).

Gel retardation assays were also performed with OccR expressed in *Agrobacterium* strain APF2(pOcc265) and the resected fragments. Figure 7C shows, as one example, the results with the promoter fragment from pOcc328 and demonstrates that there were no significant differences between the mobilities of the complexes obtained with OccR expressed in *A. tumefaciens* or *E. coli* (compare lanes 4 and 5 with lanes 7 and 8). A significant octopine-induced mobility change of the complex was not observed with pOcc328 (Fig. 7C) or with any of the resected fragments, regardless of the OccR expression in *A. tumefaciens* or *E. coli* (data not shown).

DISCUSSION

NocR and OccR belong to the LysR-type regulators by sequence similarity and type of regulation (35). Both bind to the promoters in the absence and presence of the inductors nopaline and octopine. NocR binds to a single site in the two nopaline-induced promoters, and the presence of the opine induces a small change in the footprint. Similarly small changes have been reported for OccR (36), but a comparison shows that NocR covers a larger stretch of DNA than OccR. This is particularly pronounced with Pi2/Pr[noc], for which the protected region covers the first few bases of the NocR coding region (Fig. 5). The sequence comparison indicates that the NocR binding sites of the two *noc* region promoters are very similar, and the sites compete for NocR. Both regions share motifs with the OccR binding site, and these are probably important for the binding, because OccR binds to Pi1[noc] and Pi2/Pr[noc] and NocR binds to Pi/Pr[occ] (unpublished results). LysR-type-regulated promoters contain the characteristic sequence T-N_{11}-A (10). This motif is also present in the NocR and OccR binding sites, and the presence of short inverted repeats around the conserved T and A residues appears to be typical for these promoters (Fig. 8). These results show that the regulation of the opine-induced promoters by OccR or NocR shares extensive similarities with other LysR-type regulations.

The results with the *occ* region as well as with the *noc* region indicated that the sequences flanking the binding sites for the regulators determine the extent of induction. The study of the Pi/Pr[occ] region showed that the 5′ end of the OccR coding region was necessary for optimal induction and that the absence of the 5′ end was compensated for by sequences downstream of the inducible promoter (Fig. 7A). The mechanisms of this regulation remain unclear, because the gel retardation assays revealed no binding of OccR or other *Agrobacterium* proteins to the sequences flanking the OccR binding site.

The gel retardation assays with the 433-bp promoter fragment from the *occ* region showed that octopine caused a change in the mobility of the OccR-Pi-Pr[occ] complex. This had been reported before by Wang et al. (36), who proposed that the relaxation of an OccR-induced DNA bend by the opine played an important role in the promoter activation. Interestingly, no such mobility change was observed with the resected *occ* region fragments which revealed high levels of inducible promoter activity in *Agrobacterium* strains. One possible explanation is that the extent of the activator-induced mobility change depends on the distance of the center of the DNA bend from the end of the fragments and on the length of the fragments (18) and that the resected fragments were not suitable to demonstrate the shift. However, a significant opine-induced shift was also not detected with NocR and the two *noc* region promoters Pi1[noc] and Pi2/Pr[noc], although the NocR binding sites were in or close to the center of the labelled DNA fragments. Preliminary experiments suggested that the binding of NocR induces a DNA bend (unpublished data), and our results showed that nopaline induced a change in the footprint pattern, but it remains to be investigated whether nopaline leads to significant relaxation of the bend. This question is of general interest, because it concerns one of the central mechanisms of action of LysR-type regulators.

ACKNOWLEDGMENTS

We thank K. Schnetz and B. Rak for vector pFDY180, C. Beck for vector pCB303, and G. Lurz for technical assistance.

This work was supported by Deutsche Forschungsgemeinschaft (SFB206).

REFERENCES

1. **Alt-Mörbe, J., H. Kühlmann, and J. Schröder.** 1989. Differences in induction of Ti plasmid virulence genes *vir*G and *vir*D, and continued control of *vir*D expression by four external factors. Mol. Plant-Microbe Interact. **2**:301–308.
2. **Amann, E., J. Brosius, and M. Ptashne.** 1983. Vectors bearing a

hybrid *trp-lac* promoter useful for regulated expression of cloned genes in *Escherichia coli*. Gene **25:**167–178.
3. **Byerly, K. A., M. L. Urbanowski, and G. V. Stauffer.** 1991. The MetR binding site in the *Salmonella typhimurium metH* gene: DNA sequence constraints on activation. J. Bacteriol. **173:**3547–3553.
4. **Chang, M., and I. P. Crawford.** 1989. The roles of indoleglycerol phosphate and the TrpI protein in the expression of *trpBA* from *Pseudomonas aeruginosa*. Nucleic Acids Res. **18:**979–988.
5. **Coco, W. M., R. K. Rothmel, S. Henikoff, and A. M. Chakrabarty.** 1993. Nucleotide sequence and initial functional characterization of the *clcR* gene encoding a LysR family activator of the *clcABD* chlorocatechol operon in *Pseudomonas putida*. J. Bacteriol. **175:** 417–427.
6. **Deblaere, R., B. Bytebier, H. De Greve, F. Deboeck, J. Schell, M. Van Montagu, and J. Leemans.** 1985. Efficient octopine Ti plasmid-derived vectors for *Agrobacterium*-mediated gene transfer to plants. Nucleic Acids Res. **13:**4777–4788.
7. **De Vos, G., M. De Beuckeleer, M. Van Montagu, and J. Schell.** 1981. Restriction endonuclease mapping of the octopine tumor inducing plasmid pTiAch5 of *Agrobacterium tumefaciens*. Plasmid **6:**249–253.
8. **Fried, M., and D. M. Crothers.** 1981. Equilibria and kinetics of lac repressor-operator interactions by polyacrylamide gel electrophoresis. Nucleic Acids Res. **9:**6505–6525.
9. **Galas, D. J., and A. Schmitz.** 1978. DNAase footprinting: a simple method for the detection of protein-DNA binding specificity. Nucleic Acids Res. **5:**3157–3170.
10. **Goethals, K., M. Van Montagu, and M. Holsters.** 1992. Conserved motifs in a divergent *nod* box of *Azorhizobium caulinodans* ORS571 reveal a common structure in promoters regulated by LysR-type proteins. Proc. Natl. Acad. Sci. USA **89:**1646–1650.
11. **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. USA **77:**2693–2697.
12. **Henikoff, S., G. W. Haughn, J. M. Calvo, and J. C. Wallace.** 1988. A large family of bacterial activator proteins. Proc. Natl. Acad. Sci. USA **85:**6602–6606.
13. **Hooykaas, P. J. J., and R. A. Schilperoort.** 1992. *Agrobacterium* and plant genetic engineering. Plant Mol. Biol. **19:**15–38.
14. **Huang, J., and M. A. Schell.** 1991. *In vivo* interactions of the NahR transcriptional activator with its target sequences. Inducer-mediated changes resulting in transcription activation. J. Biol. Chem. **266:**10830–10838.
15. **Hynes, M. F., R. Simon, and A. Pühler.** 1985. The development of plasmid-free strains of *Agrobacterium tumefaciens* by using incompatibility with a *Rhizobium meliloti* plasmid to eliminate pAtC58. Plasmid **13:**99–105.
16. **Kunkel, T. A.** 1985. Rapid and efficient site-specific mutagenesis without phenotypic selection. Proc. Natl. Acad. Sci. USA **82:**488–492.
17. **Kusano, T., and K. Sugawara.** 1993. Specific binding of *Thiobacillus ferrooxidans* RbcR to the intergenic sequence between the *rbc* operon and the *rbcR* gene. J. Bacteriol. **175:**1019–1025.
18. **Lane, D., P. Prentki, and M. Chandler.** 1992. Use of gel retardation to analyze protein-nucleic acid interactions. Microbiol. Rev. **56:**509–528.
19. **Lanz, T., S. Tropf, F.-J. Marner, J. Schröder, and G. Schröder.** 1991. The role of cysteines in polyketide synthases: site-directed mutagenesis of resveratrol and chalcone synthases, two key enzymes in different plant-specific pathways. J. Biol. Chem. **266:** 9971–9976.
20. **Lindquist, S., F. Lindberg, and S. Normark.** 1989. Binding of the *Citrobacter freundii* AmpR regulator to a single DNA site provides both autoregulation and activation of the inducible *ampC* β-lactamase gene. J. Bacteriol. **171:**3746–3753.
21. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193:**265–275.
22. **Nakamura, K., and M. Inouyé.** 1982. Construction of versatile expression cloning vehicles using the lipoprotein gene of *E. coli*. EMBO J. **1:**771–775.
23. **Parsek, M. R., D. L. Shinabarger, R. K. Rothmel, and A. M. Chakrabarty.** 1992. Roles of CatR and *cis,cis*-muconate in activation of the *catBC* operon, which is involved in benzoate degradation in *Pseudomonas putida*. J. Bacteriol. **174:**7798–7806.
24. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
25. **Sans, N., U. Schindler, and J. Schröder.** 1988. Ornithine cyclodeaminase from Ti plasmid C58: DNA sequence, enzyme properties, and regulation of activity by arginine. Eur. J. Biochem. **173:**123–130.
26. **Sans, N., G. Schröder, and J. Schröder.** 1987. The *noc* region of Ti plasmid C58 codes for arginase and ornithine cyclodeaminase. Eur. J. Biochem. **167:**81–87.
27. **Schindler, U., N. Sans, and J. Schröder.** 1989. Ornithine cyclodeaminase from octopine Ti plasmid Ach5: identification, DNA sequence, enzyme properties, and comparison with gene and enzyme from nopaline Ti plasmid C58. J. Bacteriol. **171:**847–854.
28. **Schneider, K., and C. F. Beck.** 1987. New expression vectors for identifying and testing signal structures for initiation and termination of transcription. Methods Enzymol. **153:**452–461.
29. **Schrell, A., J. Alt-Mörbe, T. Lanz, and J. Schröder.** 1989. Arginase of *Agrobacterium* Ti plasmid C58: DNA sequence, properties, and comparison with eucaryotic enzymes. Eur. J. Biochem. **184:**635–641.
30. **Schröder, G., J. W. S. Brown, and J. Schröder.** 1988. Molecular analysis of resveratrol synthase: cDNA, genomic clones and relationship with chalcone synthase. Eur. J. Biochem. **172:**161–169.
31. **Schröder, J., J. Von Lintig, and H. Zanker.** 1992. Catabolism of the guanidino compounds nopaline, octopine, and L-arginine in *Agrobacterium tumefaciens*: enzymes, genes, and regulation, p. 19–28. *In* P. P. De Deyn, B. Marescau, V. Stalon, and I. A. Qureshi (ed.), Guanidino compounds in biology and medicine. J. Libbey & Company Ltd., London.
32. **Simon, R., U. Priefer, and A. Pühler.** 1983. Vector plasmids for in-vivo and in-vitro manipulations of gram-negative bacteria, p. 98–106. *In* A. Pühler (ed.), Molecular genetics of the bacteria-plant interaction. Springer-Verlag, Berlin.
33. **Sung, Y.-C., and J. A. Fuchs.** 1992. The *Escherichia coli* K-12 *cyn* operon is positively regulated by a member of the *lysR* family. J. Bacteriol. **174:**3645–3650.
34. **van der Meer, J. R., A. C. J. Frijters, J. H. J. Leveau, R. I. L. Eggen, A. J. B. Zehnder, and W. M. de Vos.** 1991. Characterization of the *Pseudomonas* sp. strain P51 gene *tcbR*, LysR-type transcriptional activator of the *tcbCDEF* chlorocatechol oxidative operon, and analysis of the regulatory region. J. Bacteriol. **173:**3700–3708.
35. **von Lintig, J., H. Zanker, and J. Schröder.** 1991. Positive regulators of opine-inducible promoters in the nopaline and octopine catabolism regions of Ti plasmids. Mol. Plant-Microbe Interact. **4:**370–378.
36. **Wang, L., J. D. Helmann, and S. C. Winans.** 1992. The *A. tumefaciens* transcriptional activator OccR causes a bend at a target promoter, which is partially relaxed by a plant tumor metabolite. Cell **69:**659–667.
37. **Wek, R. C., and G. W. Hatfield.** 1988. Transcriptional activation at adjacent operators in the divergent-overlapping *ilvY* and *ilvC* promoters of *Escherichia coli*. J. Mol. Biol. **202:**643–663.
38. **Winans, S. C.** 1992. Two-way chemical signaling in *Agrobacterium*-plant interactions. Microbiol. Rev. **56:**12–31.
39. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33:**103–119.
40. **Zagursky, R. J., and M. L. Berman.** 1984. Cloning vectors that yield high levels of single-stranded DNA for rapid DNA sequencing. Gene **27:**183–191.
41. **Zambryski, P., H. Joos, C. Genetello, J. Leemans, M. Van Montagu, and J. Schell.** 1983. Ti plasmid vector for the introduction of DNA into plant cells without alteration of their normal regeneration capacity. EMBO J. **2:**2143–2150.
42. **Zambryski, P. C.** 1992. Chronicles from the *Agrobacterium*-plant cell DNA transfer story. Annu. Rev. Plant Physiol. Plant Mol. Biol. **43:**465–490.
43. **Zanker, H., J. von Lintig, and J. Schröder.** 1992. Opine transport genes in the octopine (*occ*) and nopaline (*noc*) catabolic regions in Ti plasmids of *Agrobacterium tumefaciens*. J. Bacteriol. **174:**841–849.