Identification of Transposable Elements Which Activate Gene Expression in *Pseudomonas cepacia*

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Received 30 May 1986/Accepted 3 October 1986

This study demonstrated that transposable elements in *Pseudomonas cepacia* could be inserted upstream of a poorly expressed gene and increase its expression more than 30-fold. Five elements, Tn*Pc1*, IS402, IS403, IS404, and IS405, were isolated by their ability to increase expression of the β -lactamase gene of the broad-host-range plasmid pRP1. Increased expression resulted only from insertion of these elements, suggesting that insertional activation is an important means of elevating gene expression in this organism. Four of the elements inserted between a *Pst*I site within the β -lactamase gene and a *Bam*HI site located 375 base pairs upstream of its promoter. The element IS403 inserted distal to the *Bam*HI site within the coding region for the gene *tnpR*, suggesting that insertional activation can act over greater than expected distances. In addition, the element IS402 activated the β -lactamase genes carried on plasmids pRP1 and pMR5 (temperature-sensitive pRP1) equally well in opposite orientations, demonstrating that insertional activation by this element occurs independent of its orientation.

Unlike many bacteria which are adapted to very specific nutritional niches, *Pseudomonas cepacia* can metabolize a wide variety of compounds, including many unusual growth substrates such as penicillin G (3) and octopine (W. Beckman, Ph.D. thesis, University of Massachusetts, Amherst, 1981). In addition, *P. cepacia* is both a phytopathogen, causing root rot in onion bulbs (1), and an opportunistic human pathogen (11, 14). Prior to this study, the genetic mechanisms which account for the adaptability of *P. cepacia* had not been investigated. The results presented here suggest that insertional activation by transposable elements contributes to the nutritional versatility of *P. cepacia*. As is demonstrated, transposable elements provide a means to increase the expression of a gene which *P. cepacia* acquires on a conjugative plasmid; the product of this gene is then used in the catabolism of a growth substrate. It is proposed that transposable elements enable *P. cepacia* to make use of foreign genetic material which otherwise may not be expressed. In doing so, transposable elements increase the amount of useful genetic information available to this organism and therefore contribute to its versatility.

Transposable elements were first discovered in maize by McClintock more than 30 years ago (23). Subsequently they have been found in such diverse systems as the archaebacteria (30), eubacteria (19), yeasts (6), and *Drosophila melanogaster* (26). In her pioneering work, McClintock also proposed that mobile genetic elements were involved in the regulation of gene expression (23). In support of this hypothesis, an expanding body of evidence for both procaryotic and eucaryotic systems suggests that transposable elements play roles in the positive regulation of gene expression (7, 15, 16, 18, 27, 29, 35, 36). This study describes the isolation of transposable elements from *P. cepacia* 249. It is the first

demonstration that transposable elements from this genus can activate gene expression.

MATERIALS AND METHODS

Bacterial strains and plasmids. *P. cepacia* 249 (ATCC 17616), 104 (ATCC 17478), and 383 (ATCC 17760) were obtained originally from R. Y. Stanier (33). *P. cepacia* 249-2 (pTGL6⁻), Lys⁻ Pen^s, was obtained after mutagenesis with UV light (3) and was subsequently cured of the cryptic plasmid pTGL6 (T. Beals et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, H201, p. 139). *Pseudomonas aeruginosa* PAO was obtained from P. V. Phibbs, Jr., East Carolina University Medical School. *Pseudomonas fluorescens* (ATCC 13525) was obtained from D. H. Pope, Rensselaer Polytechnic Institute, Troy, N.Y. *Pseudomonas phageolicola* HB10Y was obtained from A. K. Vidaver, University of Nebraska, Lincoln. *Escherichia coli* JA211 (RecA⁻ Leu⁻ Trp⁻) was obtained from J. Carbon, University of California, Santa Barbara. *E. coli* JC3272 (Lac F⁻ Gal⁻ Lys⁻) was obtained from S. Kaplan, University of Illinois, Champaign-Urbana.

Bacterial strains harboring plasmids pRP1 (13) and pMR5 (28) were obtained from G. Jacoby, Massachusetts General Hospital, Boston. Plasmids were transferred into strain 249 by conjugation on solid medium with selection for acquisition of tetracycline resistance.

Growth of bacteria. *Pseudomonas* cultures were grown in inorganic salts medium (1.0 mM MgSO₄, 0.1 mM CaCl₂, 0.01 mM FeSO₄, 0.2% [(wt/vol) (NH₄)₂SO₄, pH 6.5, phosphate buffer [33 mM Na₂HPO₄, 67 mM KH₂PO₄]) supplemented with the appropriate carbon source. Growth was at 37°C, except for *P. fluorescens* cultures, which were incubated at 30°C. When testing for antibiotic resistance, penicillin G and tetracycline (Sigma Chemical Co., St. Louis, Mo.) were added to 1% yeast extract medium at final concentrations of 2.0 mg/ml and 300 μ g/ml, respectively. Tests for growth on penicillin G as the sole source of carbon and energy were carried out in minimal medium supplemented with penicillin

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TABLE 1. β -Lactamase determinations^a

Strain	Mean β -lactamase sp act (nmol/min per mg of protein) $\pm$ SD	
	Without penicillin	With penicillin
<i>P. cepacia</i>		
249	33 $\pm$ 10	860 $\pm$ 60
249(pRP1)	200 $\pm$ 30	1,200 $\pm$ 170
249-2	4	— ^b
249-2(pRP1)	250	250
249-2(pTGL6 ⁻)	4 $\pm$ 0.7	— ^b
249-2(pRP1 pTGL6 ⁻)	270 $\pm$ 70	190 $\pm$ 10
249-2(pTGL51 pTGL6 ⁻)	6,800 $\pm$ 1,100	5,000 $\pm$ 730
249-2(pTGL52 pTGL6 ⁻)	7,300 $\pm$ 1,000	9,000 $\pm$ 650
249-2(pTGL53 pTGL6 ⁻)	7,300 $\pm$ 2,000	11,000
249-2(pTGL54 pTGL6 ⁻)	8,800 $\pm$ 1,200	13,000
249-2(pTGL55 pTGL6 ⁻)	11,000 $\pm$ 4,000	15,000
249-2(pMR5 pTGL6 ⁻)	160	150
249-2(pTGL62 pTGL6 ⁻)	5,700	6,200
<i>P. aeruginosa</i>		
PAO	40 $\pm$ 4	1,800 $\pm$ 200
PAO(pRP1)	2,500 $\pm$ 400	2,500 $\pm$ 300
<i>E. coli</i>		
JA211	10 $\pm$ 3	ND ^c
JA211(pRP1)	6,100 $\pm$ 200	ND ^c

^a The strains were grown in yeast extract in the absence or presence of penicillin and assayed for β -lactamase. Activities are expressed as the mean specific activity in nanomoles of cephalosporin 87/312 cleaved per minute per milligram of protein ($n \geq 4$).

^b —, No growth on penicillin medium.

^c ND, Not determined.

G at a final concentration of 20 mg/ml. *E. coli* cultures were grown at 37°C in L broth. When testing for resistance to antibiotics, penicillin G and tetracycline (Sigma) were added to the L medium at a final concentration of 20 μ g/ml.

Selection for increased production of β -lactamase. *P. cepacia* derivatives 249-2 (pRP1 pTGL6⁻) and 249-2 (pMR5 pTGL6⁻) were grown to stationary phase in 250-ml flasks containing 50 ml of 1% yeast extract medium. Bacteria were washed and suspended in 1/2 volume of sterile inorganic salts. Samples of 0.05 ml were plated on penicillin G (20 mg/ml) agar supplemented with lysine (50 μ g/ml) and incubated for approximately 72 h. Colonies which grew well were purified several times on selective medium and screened for plasmid content (21).

Assay of β -lactamase. Bacteria were grown overnight on 1% yeast extract plates with or without penicillin G (2.0 mg/ml). Cells were suspended in 0.02 M phosphate buffer, pH 6.8, cooled on ice, and disrupted by sonic treatment at 0°C for 30 s with a Branson sonifier (Branson Equipment Company, Norwalk, Conn.) equipped with a tapered microtip attachment. Unbroken cells and debris were removed by centrifugation. The supernatants were collected, placed on ice, and assayed for protein and enzyme activity. Protein concentrations were determined by the method of Lowry et al. (22). β -Lactamase activities were determined by the procedure of O'Callaghan et al. (25). Reaction mixtures contained 0.1 M phosphate buffer, pH 6.8, 10⁻⁴ M chromogenic cephalosporin 87/312 (Calbiochem), and an appropriately diluted bacterial extract in a total volume of 1.0 ml. Following a 10-min incubation at 37°C, 1.0 ml of distilled water was added and the absorbance was read at 485 nm. All β -lactamase enzyme activities are expressed as nanomoles of chromogenic cephalosporin 87/312 cleaved per minute per milligram of protein at 37°C.

Isolation of DNA. Plasmid DNA was isolated by the procedure of Currier and Nester (12). This procedure was also used to isolate bacteriophage CP1 DNA, which replicates as a covalently closed circle in *P. cepacia* 383.

For the isolation of total cellular DNA, bacteria from an overnight culture were washed in TE buffer (50 mM Tris hydrochloride, pH 8, 20 mM EDTA) and suspended in the same buffer to which 1.0 mg of type XIV protease (Sigma) per ml and 1% (wt/vol) sodium dodecyl sulfate (SDS) were added. This suspension was mixed by gently inverting the tube, followed by incubation at 37°C for a minimum of 6 h. Following cell lysis, the mixture was extracted once with an equal volume of chloroform-phenol (1:1) and once with an equal volume of chloroform-isoamyl alcohol (24:1). DNA was precipitated by adding 1/2 volume of ammonium acetate (7.5 M) and 3 volumes of 95% ethanol and incubating at -80°C for 30 min.

Restriction endonuclease analysis and DNA-DNA hybridization. Purified DNA was digested with restriction endonucleases under the conditions specified by the supplier (Bethesda Research Laboratories, Inc., Rockville, Md.). DNA fragments were transferred to GeneScreen Plus membranes (New England Nuclear Corp., Boston, Mass.) by the method of Southern (32). Membranes were prehybridized at 65°C overnight in a solution containing 0.2% (wt/vol) polyvinylpyrrolidone, 0.2% (wt/vol) Ficoll, 0.2% (wt/vol) bovine serum albumin, 1 M NaCl, 1% (wt/vol) sodium dodecyl sulfate, and denatured salmon sperm DNA (250 μ g/ml). Approximately 1 μ g of probe DNA was labeled with [³²P]dCTP (600 Ci/mol; New England Nuclear) to 3 $\times$ 10⁷ cpm/ μ g with a nick translation kit supplied by Bethesda Research Laboratories. Hybridizations were carried out in the same solution at 65°C for 24 h. Membranes were washed as outlined by the supplier. Autoradiography was performed with Kodak X-Omat XAR-5 film (Eastman Kodak, Rochester, N.Y.) and Dupont Cronex Lightning-Plus intensifying screens.

RESULTS

Expression of plasmid pRP1-encoded β -lactamase in *P. cepacia* 249. The expression of a chromosomally encoded β -lactamase gene(s) is induced when wild-type *P. cepacia* 249 is grown in the presence of penicillin (3) (Table 1). A nonreverting mutant, designated 249-2, and 249-2(pTGL6⁻), a derivative of this mutant cured of plasmid pTGL6, produced no β -lactamase (Table 1), no longer grew on penicillin G as the sole energy source, and were sensitive to this antibiotic. In an attempt to restore resistance to penicillin to 249-2 and 249-2(pTGL6⁻), the plasmid pRP1 (13) was introduced. Plasmid pRP1 is a broad-host-range conjugative plasmid which encodes resistance to penicillin on the transposable element TnI (Fig. 1). Although pRP1 conferred resistance to penicillin on 249-2 and 249-2(pTGL6⁻), it did not restore their ability to grow on penicillin. Table 1 compares the β -lactamase activity detected in extracts of these cells. *P. cepacia* 249 and 249-2(pTGL6⁻) containing pRP1 had levels of β -lactamase activity of 200 to 300 nmol/min per mg when grown in rich medium lacking penicillin, whereas levels of 2,000 to 6,000 nmol/min per mg were seen in *P. aeruginosa* and *E. coli* strains carrying pRP1. These results demonstrate that the TnI β -lactamase is expressed poorly in strain 249 and suggest that the failure of pRP1 to restore the ability to grow on penicillin on mutants such as 249-2(pTGL6⁻) is due to this poor expression.

Selection for insertions which increase β -lactamase expression. Previous to this study, a plasmid containing an inser-

tion had been identified in a spontaneous mutant of 249-2(pRP1 pTGL6⁻) (Y. N. Lee, unpublished results). *P. cepacia* 249-2(pTGL6⁻) containing this plasmid, designated pTGL51, produced 30-fold-higher levels of the plasmid-encoded β -lactamase (Table 1) and also grew on penicillin as the sole energy source. It was possible to isolate additional plasmids with insertions by selecting for derivatives of 249-2(pRP1 pTGL6⁻) which could grow on penicillin. This selection procedure was carried out six times. From 1 to 20 of the ca. 10^9 cells plated formed colonies on penicillin medium after incubation at 37°C for 3 to 6 days. In every case examined, mutants which grew on penicillin contained a plasmid with an insertion. Examination of numerous plasmids revealed four unique plasmids, which have been designated pTGL52, pTGL53, pTGL54, and pTGL55. Each of these plasmids was isolated a minimum of three times in the course of efforts to find more plasmids with insertions. This suggests that if additional insertions can occur, they do so much less frequently. β -Lactamase activity increased at least 30-fold in 249-2(pTGL6⁻) cells containing each of these plasmids (Table 1).

Each of the insertions was first characterized by size and the restriction sites it contained (Table 2). Plasmid pTGL51 contained the largest insertion, approximately 13.7 kilobases (kb). This insertion was designated Tn*PcI* to indicate that it is a transposon isolated from *P. cepacia*. Tn*PcI* was designated a transposon rather than a simple insertion sequence because of its relatively large size; however, rigorous proof for this designation will require the identification of a Tn*PcI* function unrelated to those required for transposition. The insertions in plasmids pTGL52, pTGL53, pTGL54, and pTGL55 were designated IS402, IS403, IS404, and IS405, respectively, to indicate that they are insertion sequences. These insertion sequences varied in size from 0.8 kb for IS403 to 1.5 kb for IS405.

To determine the location of the insertions on plasmid pRP1, plasmids were digested with the restriction enzyme *Pst*I. The results (Fig. 1) confirmed that the insertions were

FIG. 1. Insertions into plasmid pRP1. Plasmid pRP1 has six sites for cleavage by the restriction endonuclease *Pst*I as shown here. The 2.9-kb *Pst*I fragment within Tn*I* includes the amino-terminal coding portion and upstream sequences for the β -lactamase gene. Each of the plasmids containing insertions was compared with plasmid pRP1 after digestion with *Pst*I. Lanes: 1, pRP1; 2, pTGL51; 3, pTGL52; 4, pTGL62; 5, pTGL53; 6, pTGL54; 7, pTGL55. Plasmid digests were resolved electrophoretically on a 0.7% agarose gel in TBE buffer (89 mM Tris, 2.5 mM EDTA, 89 mM boric acid, pH 8.3). The star in lane 1 indicates the 2.9-kb fragment of pRP1 which contains an insertion in each of the plasmids from mutants which expressed increased levels of β -lactamase (lanes 2 to 6).

TABLE 2. Insertions which activate the β -lactamase gene of pRP1^a

Plasmid	Element	Size (kb)	No. of sites within element for:									No. of copies
			Bam	Cl	Eco	Hin	Kpn	Pst	Sal	Sma	Xho	
pTGL51	Tn <i>PcI</i>	13.7	3	5	4	2	1	6	6	2	5	1
pTGL52	IS402	1.1	0	1	0	0	0	0	0	1	0	4
pTGL62	IS402	1.1	0	1	0	0	0	0	0	1	0	4
pTGL53	IS403	0.8	0	0	0	1	0	0	0	1	0	10
pTGL54	IS404	1.1	0	0	0	0	0	1	1	1	0	9
pTGL55	IS405	1.5	0	1	0	0	0	0	1	0	1	9

^a The number of cleavage sites within each element is presented for the enzymes *Bam*HI, *Cl*AI, *Eco*RI, *Hin*III, *Kpn*I, *Pst*I, *Sal*I, *Sma*I, and *Xho*I. The copy numbers given represent minimum estimates of the number of copies of each element in the chromosome of *P. cepacia* 249.

in the 2.9 kb *Pst*I fragment containing the promoter and approximately 0.5 kb of the β -lactamase gene. To further localize each of the insertions, plasmids were doubly digested with a series of restriction endonucleases. This analysis localized four of the insertions to the 0.9-kb *Bam*HI-*Pst*I fragment of Tn*I* (Fig. 2A). IS403 differed from the other four in that it was located farther away from the β -lactamase gene on the other side of the *Bam*HI site, within the *tnpR* gene.

DNA-DNA hybridization experiments were performed to determine the distribution of the transposable elements in the genome of *P. cepacia* 249. There were three possible locations where the elements could reside: the chromosome, plasmid pTGL6, which is harbored in strain 249 (2), and a temperate bacteriophage, CP1, which is carried in strain 249 (9). Restriction digests of each of these DNAs were probed with radiolabeled plasmids pTGL51, pTGL52, pTGL53, pTGL54, and pTGL55. The minimum number of copies of each element in strain 249 was estimated by probing two samples of genomic DNA, one which had been cleaved with an enzyme which did not cut within a given element and a second which had been cleaved with an enzyme which cut once within the element. The results for IS404 and IS405 are shown in Fig. 3, and the minimum estimates of the copy number of each of the elements in the genome of *P. cepacia* are summarized in Table 2. All of the elements were found to reside on the chromosome of strain 249. Although the elements IS404 and IS405 differed in length and in the sites for several restriction enzymes (Table 2), they hybridized to identical fragments of genomic DNA digested with several different restriction enzymes. Plasmid pTGL6 harbored one copy of an element homologous to IS404 and IS405 as well as one copy of IS402. Two copies of IS404 or IS405 were carried on the temperate bacteriophage CP1 (data not shown).

Each of the plasmids also was used as a probe in a preliminary survey to determine whether copies of homologous elements were present in other pseudomonads or *E. coli*. Genomic DNAs from *P. cepacia* 104, *P. aeruginosa* PAO, *P. fluorescens* 13525, *P. phaseolicola* HB10Y, and *E. coli* JC3272 were isolated and cleaved with *Eco*RI. The elements IS404 and IS405 hybridized to nine *Eco*RI fragments of total genomic DNA from *E. coli* JC3272 (Fig. 4). No homologies between the genomes of the other gram-negative bacteria tested or any of the *P. cepacia* elements were detected.

Insertional activation by IS402 in either orientation. Plasmid pMR5 is virtually identical to plasmid pRP1, but due to a temperature-sensitive mutation pMR5 is unable to replicate at higher temperatures (28). As expected, the β -lactamase gene carried on pMR5 was also poorly expressed

FIG. 2. Localization of insertions within TnI. (A) Schematic drawing of the TnI region of plasmid pRP1. The genes encoding the transposase (*tnpA*), the resolvase (*tnpR*), and the β -lactamase (*bla*) of TnI, as well as the inverted repeats (IR-L and IR-R) at the ends of the element, are shown. In addition, the fragments generated by cleavage of this region with *Pst*I and *Bam*HI are presented. The hatched area represents the 0.9-kb *Bam*HI-*Pst*I fragment which contains the insertions in plasmids pTGL51, pTGL52, pTGL62, pTGL54, and pTGL55. The insertion in plasmid pTGL53 was in the 1.9-kb *Bam*HI-*Pst*I fragment, within the *tnpR* gene. (B) Enlargement of the TnI region which contains an insertion of IS402 in plasmids pTGL52 and pTGL62. The sites for the enzymes *Sma*I and *Cla*I, which cleave within IS402, are shown. The distribution of these sites was determined by double digestion of plasmids with *Bam*HI-*Cla*I and with *Bam*HI-*Sma*I. The results indicate that IS402 is inserted in opposite orientations in plasmids pTGL52 and pTGL62.

in *P. cepacia* 249 (Table 1). Selection for better expression resulted in the isolation of a derivative of plasmid pMR5, designated pTGL62, containing an insertion. Cells containing pTGL62 produced 30-fold more β -lactamase in a manner analogous to that seen for the activation of the β -lactamase gene of pRP1 (Table 1). Analysis of plasmid pTGL62 with a variety of restriction endonucleases demonstrated that the insertion in pTGL62 was identical in size and restriction sites to IS402, which was present in pTGL52 (Fig. 1, Table 2).

The insertion in pTGL62 was localized to the same 0.9-kb *Bam*HI-*Pst*I fragment of TnI (Fig. 2A) that contained IS402 in pTGL52. In addition, Southern hybridization experiments demonstrated that the insertions in plasmids pTGL52 and pTGL62 were homologous (data not shown). It was determined that the insertions in these two plasmids were in opposite orientations by double digestion with an enzyme

FIG. 3. Hybridization of IS404 and IS405 to sequences from *P. cepacia* 249. The autoradiographs show hybridization of the pTGL54 (A) and pTGL55 (B) probes to DNA sequences from the genome of strain 249. In both cases, the probes hybridized strongly to an 11.5-kb *Eco*RI fragment of pTGL6, as well as to nine *Eco*RI fragments of total genomic DNA. Lanes: 1, pTGL54 and pTGL55 cut with *Sma*I; 2, pTGL6 cut with *Eco*RI; 3, total genomic DNA cut with *Eco*RI.

FIG. 4. Hybridization of IS404 and IS405 to sequences from *E. coli* JC3272. The autoradiographs show hybridization of the pTGL54 (A) and pTGL55 (B) probes to sequences from the genome of *E. coli* JC3272. Both probes hybridized strongly to the same nine *Eco*RI fragments of total genomic DNA. The lanes contained total genomic DNA from *E. coli* JC3272 digested with *Eco*RI.

which cleaved asymmetrically within IS402, either *Sma*I or *Cla*I in combination with *Bam*HI. The results of this analysis (Fig. 2B) demonstrated that the insertion of IS402 in pTGL62 was in opposite orientation to the insertion in pTGL52.

DISCUSSION

The results presented here identify five transposable elements in *P. cepacia* which are capable of insertionally activating a poorly expressed β -lactamase gene. In every case, the activation was 30-fold or greater. It appears that insertional activation is a common means of increasing gene expression in *P. cepacia* because all of the mutations which we identified as increasing β -lactamase expression were insertions. Although the results of previous investigations (10, 34) confirmed the existence of transposable elements in *Pseudomonas* species, this is the first demonstration of their ability to activate gene expression.

We were able to select for insertional activation of the β -lactamase gene of pRP1 because activation allowed mutants of *P. cepacia* to grow on penicillin. In analogous experiments we also observed insertional activation of a β -galactosidase gene which was introduced into *P. cepacia* on a broad-host-range plasmid (G. E. Scordilis, Ph.D. thesis, University of Massachusetts, Amherst, 1985). In this case, increased production of β -galactosidase allowed *P. cepacia* to grow on lactose. Together, these observations suggest a role for transposable elements in contributing to the versatility of *P. cepacia* 249. Foreign genes which *P. cepacia* acquires from other gram-negative bacteria, for example via conjugation of broad-host-range plasmids, are not always well expressed. Transposable elements provide *P. cepacia* with an efficient way of increasing the expression of a gene. Under the appropriate conditions, the products of insertionally activated genes can be used in the catabolism of a substrate. It is therefore reasonable to hypothesize that transposable elements in *P. cepacia* function to increase the pool of useful genetic information and, in doing so, contribute to its metabolic diversity.

It was of interest to determine whether sequences homologous to the *P. cepacia* elements were present in other less metabolically diverse gram-negative bacteria. We did not detect homologies to the *P. cepacia* elements in any of the other *Pseudomonas* strains tested here. The elements IS404 and IS405, however, did hybridize to nine *Eco*RI fragments of total genomic DNA from *E. coli* JC3272. It is somewhat surprising that these elements had homology to sequences in *E. coli* and not to sequences from more closely related *Pseudomonas* species. However, an earlier study reported that sequences homologous to the *E. coli* insertion sequence IS2 could be detected in the genomic DNA from strains of *P. aeruginosa* and *P. putida*, supporting the notion that these two genera have homologous repetitive sequences (24).

This study is the first demonstration of the activation of gene expression by transposable elements in a *Pseudomonas* species; however, this phenomenon has been well documented in both eucaryotes (15, 23, 31, 35) and *E. coli* (4, 5, 8, 16, 17, 20, 27, 29, 36). Studies on *E. coli* have suggested mechanisms for the activation of gene expression by transposable elements. Reynolds et al. (27) demonstrated that the insertion of IS1 or IS5 at a distance of 90 to 130 base pairs from the site of transcription initiation activated expression of the cryptic *bgl* operon of *E. coli*. They suggest that activation is a result of a change in DNA conformation. Jaurin and Normark (18) demonstrated that the insertion of IS2 in one orientation next to the *ampC* gene created a

hybrid promoter consisting of a -35 region from IS2 and the -10 region of *ampC*. Better expression is due to the increase in distance between the -35 and -10 regions from 16 base pairs in the *ampC* promoter to 17 base pairs in the *ampC*-IS2 hybrid promoter (18). In the case of *argE* activation by IS3, Charlier et al. (7) demonstrated that transcription of the activated *argE* gene was initiated 44 nucleotides within IS3. They concluded that IS3 carries a promoter at one of its ends.

The activation of gene expression by transposable elements in *P. cepacia* and *E. coli* may be similar; however, the mechanism(s) of activation in *P. cepacia* has not yet been defined. The results of this study point to some potentially important differences. Restriction analysis and DNA-DNA hybridization experiments comparing plasmids pTGL52 and pTGL62 support the hypothesis that the same element, IS402, is inserted in opposite orientation in these plasmids. In both pTGL52 and pTGL62, the insertion increased β -lactamase production approximately 30-fold. It appears, therefore, that IS402 is capable of activating β -lactamase expression equally well in either orientation. In addition, the element IS403 activated β -lactamase expression by inserting relatively far upstream (>700 nucleotides) of the normal start for transcription of the β -lactamase gene. Given the precedents set by *E. coli*, both of these findings were surprising. It remains to be seen whether they reflect significant differences between the mechanisms of activation in these two organisms. More importantly, an investigation of the mechanisms of gene activation by the *P. cepacia* elements will provide important information about the regulation of gene expression in pseudomonads. At present a thorough investigation of this has not been carried out for members of this diverse genus.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant GM-21198 from the National Institutes of Health and by grant DMB-84128 from the National Science Foundation.

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