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Alteration of the curved helical structure located in the upstream region of the β-lactamase promoter of plasmid pUC19 and its effect on transcription

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ABSTRACT

The region preceding the β-lactamase promoter of *Escherichia coli* plasmid pUC19 has a curved DNA (bent DNA) structure. The center of the curvature was revealed to exist around nucleotide position 2580 of the plasmid, which is just beside RNA polymerase binding region. It was indicated that the identified region is curved even at 60°C. The gross geometry of the curvature was altered by inserting synthetic double-stranded oligonucleotides between positions 2585 and 2586. Effect of the alteration on strength of the promoter was not detected *in vitro*. However, *in vivo* analyses showed that the promoter strength is apparently dependent, in part, on the gross geometry of the curvature. Insertions of 4 and 16 bp, both of which altered the gross geometry of the curvature greatly, caused considerable reductions of *in vivo* level of β-lactamase mRNA. *In vivo*, overall three-dimensional structure of the region covering the promoter and the curvature seems to play some significant role in transcription of the gene.

INTRODUCTION

Tertiary structures of DNA have been shown to be sensitive to nucleotide sequence. Repetition of adenine (thymine) tracts with a spacing close to the helix pitch can cause intrinsic bending of DNA. Curved DNA was first suggested to exist in a fragment from *Leishmania tarentolae* kinetoplast DNA rich in adenine tracts (1). This fragment showed anomalously retarded migration in nondenaturing polyacrylamide gels. Later, direct evidences for the existence of curved helical structure were provided by, for example, an electron microscopic study (2) and circularization experiments of DNA fragments (3,4). To date, numerous examples of naturally occurring curved DNA have been reported based on the analyses of altered electrophoretic mobility of DNA fragments. Many of them have been found in biologically important regions in genomes such as those involved in the initiation of DNA replication, control of transcription and site-specific recombination (reviewed in 5,6), alluding to some role of such unusual structures in essential cellular events.

In both prokaryotic and eukaryotic cells, transcriptional control regions of several genes have been reported to have a curved DNA structure (for example: 7–14). Furthermore, some data illustrating the significance of curved DNA structure for efficient transcription in prokaryotes were reported recently (11, 15–20). However, for a clear understanding of the significance of DNA curvature, more extensive knowledge of both structural features and functions seems to be still necessary.

DNA fragments containing the β-lactamase promoter region of plasmid pBR322 have been reported to show anomalously slow electrophoretic mobility in nondenaturing polyacrylamide gels (21). However, the locus responsible for the anomaly has not been determined precisely. Recently, a better map of the locus was presented by an electron microscopic study (22). It was shown that the region spanning nucleotides 4200–50 has a curvature. Transcription of the β-lactamase gene starts at nucleotide number 4189 of the plasmid and the number 4200 corresponds to the sequence position −11 relative to the start site. In this study, the role of the corresponding curvature of pUC19 in transcription of the β-lactamase gene was investigated. First, on the basis of the studies described above, the center of the curvature was determined precisely. Then, synthetic double-stranded oligonucleotides of various lengths were inserted into a site located very close to the center, and finally, the influence of the altered DNA conformation on transcription was investigated *in vitro* and *in vivo*. It became apparent that *in vivo* transcription from the β-lactamase promoter is dependent, in part, on the gross geometry of the curvature.

MATERIALS AND METHODS

Materials

Restriction endonucleases and linkers were obtained from Takara Shuzo, Toyobo, or from New England Biolabs. *E. coli* DNA polymerase I large (Klenow) fragment, T4 DNA polymerase, T4 DNA ligase, T4 polynucleotide kinase, *E. coli* alkaline phosphatase (BAP) and sequencing reagents were from Takara Shuzo. pUC19 DNA (23) and phage ΦX174 DNA digested with *Hin*cII or *Hae*III (size marker) were from Toyobo. *E. coli* RNA polymerase holoenzyme was purchased from Boehringer Mannheim. MMLV reverse transcriptase was from Life Technologies, Inc.. [α-^{32}P]GTP and [γ-^{32}P]ATP (3000 Ci/mmol each) were from Amersham and NEN Research Products respectively. All other reagents were obtained from Sigma, Nakarai Chemicals, or from Wako Pure Chemicals.

Bacterial strain

E. coli strain HB101 cells were used as transformation hosts for plasmids.

Permutation analysis

The permutation analysis was carried out as described by Wu and Crothers (24). DNA fragments used for the analysis were prepared as follows. First, we inserted the 380 bp *Bbi*II-*Bbi*II fragment from pUC19 (spanning nucleotides 2235−2617) into the unique *Aat*II site (position 2617) of another pUC19 by blunt-end ligation (nucleotide sequence numbers designate the first base pair present on the recognition site of each restriction enzyme in this paper). Then, a construct carrying the *Bbi*II-*Bbi*II region as a tandem dimer was selected. This plasmid (pBL2, 3066 bp) was then digested with *Sca*I and *Eco*0109I and a DNA fragment carrying the dimer was isolated through agarose gel electrophoresis (a *Sca*I-*Eco*0109I fragment of 876 bp). To produce a set of permuted fragments, this fragment was further digested with *Apa*LI, *Bsp*HI, *Dra*I, *Fnu*4HI or *Tth*HB8I, respectively. Each resulting digest was phenol-extracted, ethanol-precipitated, rinsed with 70% ethanol and dried. As the *Sca*I-*Eco*0109I fragment was not easily digested with *Ssp*I for unknown reasons, we digested pBL2 with *Ssp*I directly and then purified the 381 bp fragment.

Nondenaturing polyacrylamide gel electrophoresis

7.5% polyacrylamide gel {acrylamide/bisacrylamide = 29/1 (wt/wt)} electrophoreses were performed in 45 mM Tris-borate (pH 8.3) and 1 mM EDTA. Unless otherwise indicated, these were carried out at 2.4 V cm^{-1} at 4.5°C. After electrophoreses, gels were stained with ethidium bromide.

Constructions of variant plasmids

Plasmid pX16 was constructed as follows. First, the 493 bp *Apa*LI fragment (nucleotides 2366−177) was extracted from plasmid pUC19, treated with BAP, and digested with *Nla*IV (recognition site at 2583). Then, a 16 bp non-phosphorylated *Sca*I linker (5′-GTCGCAGTACTGCGAC-3′) was ligated within the site. The ligation product (mutant *Apa*LI fragment) was ligated again with the other *Apa*LI fragments of pUC19, and the resulting ligation mixture was used to transform cells of *E. coli* strain HB101. Ampicillin-resistant colonies were screened by digestion with restriction enzymes.

Plasmid pX10 has a 10 bp fragment (5′-GGACTGCGAC-3′) inserted into the same *Nla*IV site. It was constructed as follows. First, an *Apa*I linker (5′-GGGGCCCC-3′) was ligated to the *Nla*IV end of the 523 bp *Ava*II-*Nla*IV fragment from pUC19 (nucleotides 2059−2583). Then, the ligation product was digested with *Sca*I (recognition site at 2177) and *Apa*I, and treated with T4 DNA polymerase to eliminate 3′ overhang generated by *Apa*I digestion. The resulting *Sca*I-*Apa*I (blunt) fragment was ligated to the 2288 bp *Sca*I-*Sca*I fragment derived from pX16 (*Sca*I digestion of pX16 generates 414 and 2288 bp fragments). Transformation of *E. coli* and screening of ampicillin-resistant colonies were carried out as described above.

Plasmid pX04 bears a 4 bp insert (5′-CGAC-3′). This was obtained as an unexpected product when a subcloning was carried out to join the *Sca*I-*Nla*IV fragment from pUC19 (nucleotides 2177−2583) with the 2288 bp *Sca*I-*Sca*I fragment from pX16.

Sequence of each construct was verified according to the dideoxy procedure.

In vitro transcription

Template DNAs were obtained from pUC19 DNA or derivatives of pUC19 described above. Digestion of the plasmids with *Dra*I and *Eco*0109I produced templates to be tested for promoter activity (test templates, corresponding to nucleotides 2274−2674 of pUC19). A template providing the reference signal for the transcription reaction was produced by digesting pUC19 with *Apa*LI and *Eco*0109I (reference template, nucleotides 2366−2674).

Reaction mixtures contained 7 nM *E. coli* RNA polymerase, 4 nM test template, 8 nM reference template, 40 mM Tris-HCl (pH 8.0), 8 mM $MgCl_2$, 150 mM KCl, 0.1 mM dithiothreitol, 0.1 mM EDTA, 0.15 mM each of ATP, GTP, UTP, and CTP, and 20 μCi of [α-^{32}P]GTP. The reactions were initiated by addition of RNA polymerase and carried out at 37°C. At given intervals, aliquots were withdrawn and transferred into 400 μl of solution containing 7 M urea, 350 mM NaCl, 10 mM Tris-HCl (pH 8.0), 10 mM EDTA, 0.5% SDS, and 2.5 μg/ml tRNA. The transcripts were extracted with phenol, chloroform, ethanol-precipitated twice, and then resolved by 6% polyacrylamide gels {acrylamide/bisacrylamide = 29/1 (wt/wt)} containing 7 M urea. Transcription levels were determined using the Fujix BAS2000 Bio-image analyser (Fuji Photo Film Co., Ltd.).

Analysis of ampicillin resistance

HB101 cells harboring pUC19, pX04, pX10, or pX16 were grown overnight in LB-broth containing 50 μg/ml ampicillin at 37°C. Then, each culture was diluted 100-fold into fresh medium containing 50 μg/ml ampicillin and the cells were grown again to A_{550} = 0.4. A part of each culture was then diluted 10^6-fold into LB-broth in order to plate cells on LB agar plates containing 25 μg/ml, 3 mg/ml, or 5 mg/ml ampicillin. Then, 150 μl of each diluted culture was plated at the three ampicillin concentrations and incubated at 37°C. After 24 hours, the number of colonies formed on each plate was counted. The remainders of the undiluted cultures (A_{550} = 0.4) were used for nucleic acids isolation.

mRNA analysis

Using cells described above, high-temperature phenol extraction was used for mRNA isolation. Amounts of β-lactamase mRNA synthesized *in vivo* were determined by the primer extension technique. Each reaction contained 1.7 μg of nucleic acids sample. 5′-^{32}P labeled oligonucleotide of the sequence 5′-GAC-ACGGAAATGTTGAATACTCATACTCTT-3′ was used as a primer. Condition used for the reaction was that recommended by the supplier of MMLV reverse transcriptase. The reaction products were resolved by PAGE {8% polyacrylamide gel (acrylamide/ bisacrylamide = 29/1), 7 M urea}. Under the reaction condition, the intensity of the extended chain was found to be proportional to the amount of total nucleic acids used in the hybridization.

RESULTS AND DISCUSSION

Permutation analysis

It is expected that alteration of a curved DNA conformation can be easily achieved by inserting short oligonucleotides into any site located within the center region of the curvature. In order to determine the target site for the insertion, the center of the curvature located in the upstream region of the β-lactamase

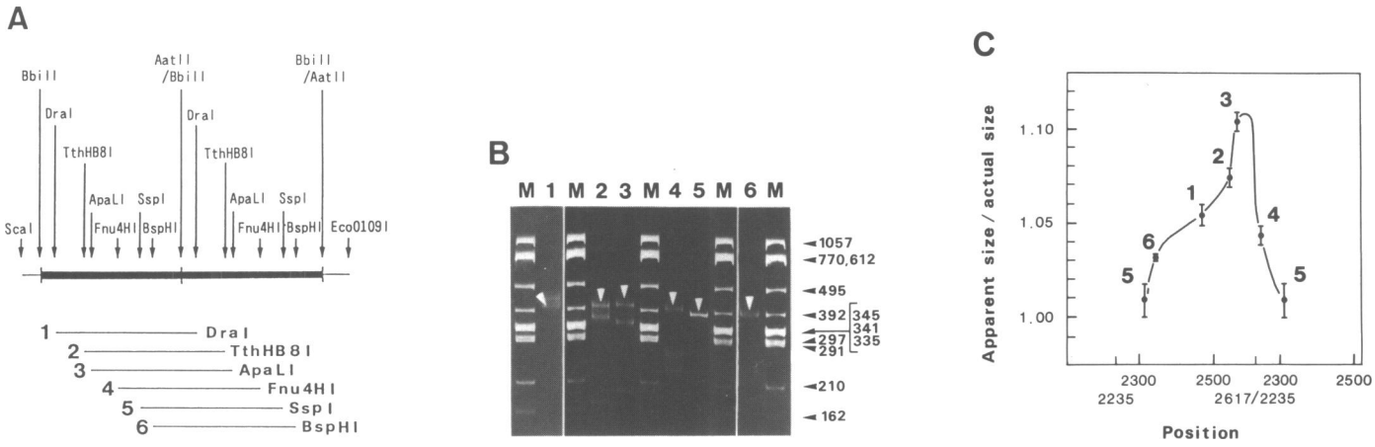


Figure 1. Permutation analysis of a fragment carrying the β-lactamase promoter region. (A) DNA fragments used for the analysis. Each permuted fragment is shown below the restriction map and the fragment is numbered on the left and named by the enzyme used to produce it on the right. Except for fragment 5, each fragment was obtained by digesting the *Sca*I-*Eco*0109I fragment with the respective enzyme (see MATERIALS AND METHODS). (B) Electrophoresis of the permuted fragments. Lanes are indicated according to the numbers in (A). Lanes M indicate marker DNA fragments (*Hin*cII digest of phage ΦX174 DNA). White arrow heads indicate the fragments of interest. (C) Mapping of the curved DNA center. Relative sizes of the permuted fragments are plotted against the centers of the respective fragments. Values are means ± S.E. of three separate experiments.

promoter of pUC19 was investigated by the permutation analysis devised by Wu and Crothers (24). For the analysis, we constructed a plasmid pBL2 which carries the 380 bp *Bbi*II-*Bbi*II region of pUC19 (containing the β-lactamase promoter region) as a tandem dimer (see MATERIALS AND METHODS). First, the *Sca*I-*Eco*0109I fragment (876 bp), which contains this duplicated region, was obtained from pBL2. Subsequently, this fragment was digested with several restriction endonucleases which cut each unit of the dimer uniquely to yield a set of permuted fragments of about 380 bp (Fig. 1A). The restriction endonucleases employed were *Apa*LI, *Bsp*HI, *Dra*I, *Fnu*4HI, *Ssp*I and *Tth*HB8I. Fig. 1B shows electrophoretic behaviors of the permuted fragments (indicated by white arrow heads). The permuted fragment yielded by digestion with *Apa*LI (377 bp) showed the slowest migration (lane 3). It behaved like a 420 bp fragment. Relative size (RS) of the fragment, which is calculated as the apparent size of the fragment divided by its actual size, was 1.11. This value is not high. Under the same electrophoretic conditions, for example, the curved DNA from human adenovirus type 2 enhancer gave RS values of 1.7−1.8 (14), which are not especially high values among curved DNA fragments. The reason for the low value (1.11) is that a coiled structure (a small part of a right-handed coil) appears to be formed (discussed later). A coiled molecule moves faster through a gel than a plane curve of the same length, owing to its smaller cross-sectional area (25). The relationship between the permuted fragments and their RS values is drawn in Fig. 1C (note that each RS value is plotted against the center of each permuted fragment). In the permutation analysis, the peak of plotted curve indicates the center region of a DNA curvature. It is about nucleotide position 2580 in this case.

Nucleotide sequence around the curved DNA center

The nucleotide sequence around the center is shown in Fig. 2. $(T\cdot A)_n$ runs and $(A\cdot T)_n$ runs are indicated by arrow heads. Both of them seem to be the principal determinants of the curved DNA

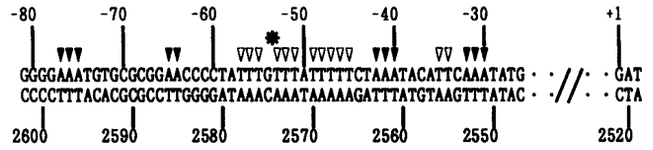


Figure 2. Nucleotide sequence of the curved DNA region. Arrow heads indicate $(TA)_n$ or $(AT)_n$ runs supposed to be the principal determinants of the curvature. The putative curved center is marked with an asterisk. Numbering above the sequence is relative to the transcription initiation site of the β-lactamase gene, +1 (26).

structure. In addition, if 10.5 base pairs are counted off in each direction from the position between nucleotides 2574 and 2575, a remarkable symmetry of $(A\cdot T)_{2-3}$ runs is observed with such runs occurring very close to positions 10.5 and 21 in both directions. This good phasing of $(A\cdot T)_{2-3}$ runs seems to play a considerable part in the formation of the curved DNA structure. The runs of A or T in this region lie at a mean helical repeat of 11.3 bp (76−31 = 45 bp, 45/4 = 11.3), versus a twist repeat in the DNA of ca. 10.4 bp. So the molecule presumably forms a small part of a right-handed coil (25). As described above, this well explains the unexpectedly low RS value of the permuted *Apa*LI-*Apa*LI fragment. Interestingly, a $(A\cdot T)_5$ stretch spanning nucleotides 2656−2660 (4324−4328 of pBR322) has been suggested to form a slightly curved DNA conformation (27). Thus, the extended region preceding the −35 region of the β-lactamase promoter contains several representative elements of DNA curvatures.

Transcription of the β-lactamase gene starts at nucleotide 2521 of the plasmid (nucleotide 4189 of pBR322) (26,28), about 54 bp downstream from the center of the symmetry (the putative curved center). This center exists just beside RNA polymerase binding region (about −50 ±20) which was previously determined by DNaseI footprinting assay (26).

Property of the curvature

Anomalous electrophoretic behaviors observed for curved DNA fragments are known to be diminished at higher temperature (29–31). The temperature dependence of relative size was also tested using the permuted *Apa*LI-*Apa*LI fragment which had shown the slowest migration among the permuted fragments. The electrophoretic anomaly observed for the fragment changed in a temperature dependent manner, thus displaying a property of curved DNAs (Fig. 3). However, as shown in the figure, a slight anomaly was still observed even at 60°C. Furthermore, the retarded migration observed at 4.5°C was almost maintained at 47°C. These phenomena might be explained in terms of the changing planarity of the curvature due to the temperature dependence of the DNA twist angles, or also explained by assuming that this DNA curvature might be considerably stiff. However, at present, we cannot conclude which explanation is more to the point. In either case, the curved DNA conformation does not seem to disappear under 37°C. *In vitro* transcription reactions were carried out at this temperature.

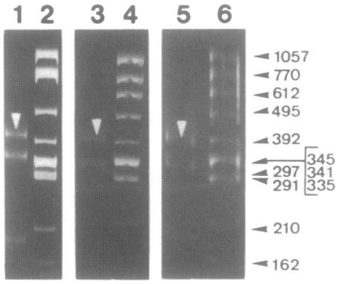
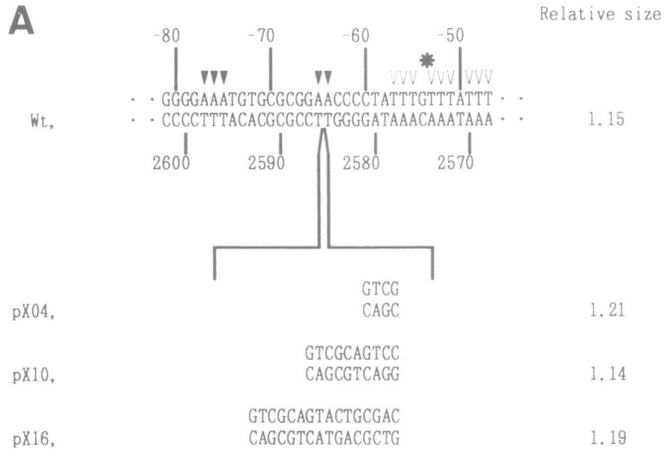


Figure 3. Electrophoretic mobilities of the permuted *Apa*LI-*Apa*LI fragment at higher temperature. *Apa*LI digest of the *Sca*I-*Eco*0109I fragment (see Fig. 1A) was used for the electrophoreses. Electrophoreses were performed at 47°C (lanes 3 and 4) and at 60°C (lanes 5 and 6). The result at 4.5°C (in Fig. 1B) is shown again for comparison (lanes 1 and 2). Lanes 2, 4 and 6 are markers (*Hin*cII digest of ΦX174 DNA). White arrow heads represent the permuted fragment.



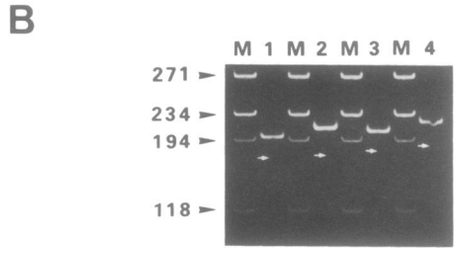


Figure 4. Alteration of the gross geometry of the curvature. (A) Nucleotide sequences of the mutant plasmids constructed. (B) Electrophoretic behaviors of restriction fragments containing the relevant region. Lane 1, pUC19 (wild-type); 2, pX04; 3, pX10; 4, pX16; M, marker DNA fragments (*Hae*III digest of ΦX174 DNA). Plasmid DNAs were digested with *Ssp*I and *Eco*0109I. The resulting products were extracted with phenol, washed with ether, precipitated with ethanol, and then electrophoresed. The expected mobilities of the fragments are indicated by white arrows {pUC19, 172 bp (nucleotides 2501–2674); pX04, 176 bp; pX10, 182 bp; pX16, 188 bp}. Relative sizes of the fragments are shown in (A).

Alteration of DNA conformation of the curvature

To alter the gross geometry of the curvature, synthetic double-stranded oligonucleotides of various lengths were inserted into a *Nla*IV site located 9 bp upstream from the putative curved center (Fig. 4). This site was chosen because it exists very close to the curved center and it is not involved in *E. coli* RNA polymerase binding region (about −50 ±20) (26). In short, we proposed to alter DNA conformation of the curvature greatly without changing DNA structure of the RNA polymerase binding region.

The plasmids pX04, pX10 and pX16 were constructed for the purpose (Fig. 4A). The inserts in them rotated the upstream half of the curved DNA around the helix with respect to the RNA polymerase binding region (the 4, 10, and 16 bp inserts generate rotational displacements of 138°, 346°, and 194° respectively). In addition, these inserts also increased the distance between the two regions.

Electrophoretic behaviors of the relevant *Ssp*I-*Eco*0109I fragments are shown in Fig. 4B and their RS values in Fig. 4A. The original curved center (indicated in Fig. 4A) is located near the center of each of the altered fragments. The RS values of the fragments derived from pX04 and pX16 were higher than the values of the fragments from Wt (wild-type, pUC19) and pX10. By reference to the theoretical study of Calladine *et al.* (25), this result is explained as follows: the inserts made new curvature repeats of (45 + 4)/5 = 9.8, (45 + 10)/5 = 11.0, or (45 + 16)/6 = 10.2 bp; (i)the 4 bp insert moved more slowly in the gel, since 9.8 is closer to the twist repeat in the DNA of

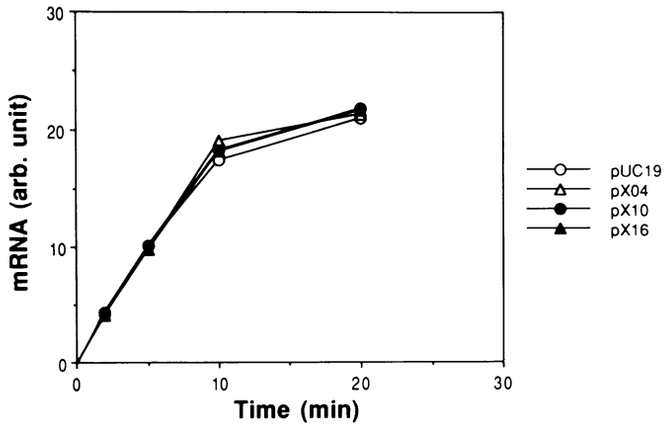


Figure 5. *In vitro* transcription analysis of mutant templates. Transcription was carried out as described in MATERIALS AND METHODS. Values are means of three separate experiments.

ca. 10.4 bp than the original mean helical repeat of 11.3 bp; (ii)the 10 bp insert moved about the same, since 11.0 is close to the original 11.3; and (iii)the 16 bp insert moved more slowly again, because 10.2 is closer to ca. 10.4 than 11.3. These numerals also indicate that the three-dimensional architectures of the curvatures of pUC19 and pX10, and those of pX04 and pX16 seem to be similar with each other, respectively (pUC19 and pX10, right-handed coils; pX04 and pX16, 'plane curves'). Similarities in the RS values in Fig. 4A {1.15 (pUC19) and 1.14 (pX10), 1.21 (pX04) and 1.19 (pX16)} seem to support this idea experimentally.

We had also tried to construct some mutants harboring other inserts. However, we could not succeed ultimately. Unfavorable modifications of the original curvature might not be tolerated by the cells.

Relative *in vitro* transcriptional activity of the constructs

Effects of the mutant upstream regions on *in vitro* transcription of the β-lactamase gene were investigated. As shown in Fig. 5, the insertions produced neither positive effect nor negative effect. This result did not depend on the concentration of *E. coli* RNA polymerase (not shown). We could not detect any effect in a preliminary experiment using supercoiled templates, also. In addition, an equivalent result was obtained when single round transcription assays were performed by employing heparin (not shown). Thus, alteration of the curved DNA conformation did not influence *in vitro* transcription by the RNA polymerase.

Effects of the insertions on *in vivo* activity of the β-lactamase promoter

Resistance of HB101 cells harboring each mutant plasmid to ampicillin on plates was tested (Fig. 6). Although a difference in the resistance between these cells and control cells (harboring pUC19) was not obvious under the conditions containing 25 μg/ml −3 mg/ml ampicillin, a marked difference was observed at the concentration of 5 mg/ml, where compared with the control cells, the cells harboring mutant plasmids showed reduced levels of the resistance in an insertion length dependent manner. Among the insertions, the 10 bp insertion (about one helical turn of B-DNA), which almost maintained the original rotational orientation of the upstream half of the original curvature relative to the RNA polymerase binding region but caused a linear displacement of the half, generated the plasmid that endowed *E. coli* cells with the strongest resistance to ampicillin on plates (pX10). On the other hand, the 4 and 16 bp insertions (about 0.5 and 1.5 helical turns), both of which caused both rotational and linear displacements of the half and contributed to the formation of stronger curvatures (plane curves) as discussed above, resulted in extremely reduced resistances (pX04, pX16). Furthermore, in these two cases, sizes of the colonies formed on plates were very small as compared with the cases of pUC19 and pX10 (not shown).

To verify that the difference in the ampicillin resistance of the *E. coli* cells was caused by a difference in the amount of β-lactamase mRNA synthesized *in vivo*, the *in vivo* mRNA levels were investigated (Fig. 7). As shown in the figure, all insertions were revealed to reduce the amount of β-lactamase mRNA *in vivo*. Also revealed was that the mRNA levels changed in an insertion length dependent manner, just like the changes in ampicillin resistance shown in Fig. 6 (data at 5 mg/ml). Figure 7 clearly shows that among the insertions, the 10 bp insertion had the least influence on the transcription (lane pX10). Amounts of β-lactamase mRNA derived from pX04, pX10 and pX16 were quantitated to be 57%, 67% and 57% of that from pUC19, respectively. Furthermore, from analyses of plasmid yield, it was ascertained that each variant plasmid and pUC19 have almost the same copy number in *E. coli* strain HB101 (not shown). Thus, it became evident that the difference in the *in vivo* mRNA levels is responsible for the difference in the ampicillin resistance of *E. coli* cells. Considering that conformations of the curvatures of pUC19 and pX10, and those of pX04 and pX16 seem to be similar with each other, respectively (pUC19 and pX10, right-handed coils; pX04 and pX16, plane curves), and that the mRNA level of pX10 was higher than those of pX04 and pX16 (1.18-fold), it is apparent that *in vivo* transcription from the β-lactamase promoter is dependent, in part, on the gross geometry of the DNA curvature.

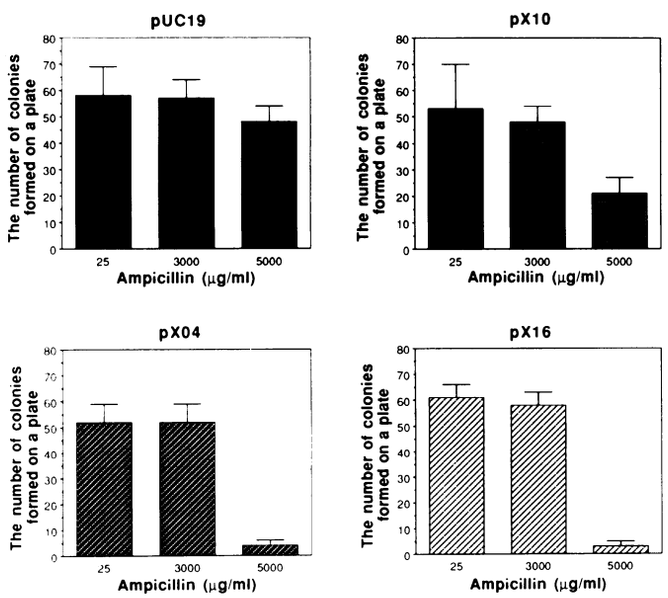


Figure 6. Resistances of the HB101 cells harboring pUC19, pX04, pX10, or pX16 to ampicillin on plates. Values represent means ± S.E. of quadruplicate or quintuplicate determinations from a representative experiment.

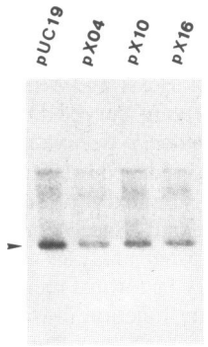


Figure 7. Analysis of the *in vivo* mRNA levels. Primer extension analysis was carried out as described in MATERIALS AND METHODS. Position of the primer-extended DNA fragment showing amounts of the correctly initiated β-lactamase mRNA is indicated by an arrowhead.

Amouyal and Buc showed that RNA polymerase prefers negative change in linking number and negative writhing, in other words a left-handed coil (32). Taking the report into consideration, we can conceive that a right-handed coil is not expected to bind RNA polymerase. In the transcription of the β-lactamase gene, some auxiliary factor might interact with the small part of a right-handed coil and stimulate the transcription. However, we can also raise the following possibilities: (i)a small part of a right-handed coil stimulates transcription by some unknown means; (ii)a right-handed coil has no effect on transcription, but a plane curve in the wrong direction disturbs action of RNA polymerase to some extent. These possibilities are being examined.

Our result is similar to the results reported by McAllister and Achberger (11), Bracco *et al.* (15), and Collis *et al.* (16) in essence. McAllister and Achberger studied function of the curved DNA located in the upstream region of the Alu156 promoter of the *Bacillus subtilis* bacteriophage SP82 (11). In their study, 11 and 21 bp insertions (about one and two helical turns) between the curvature and the −35 region of the promoter retained most of the activity of the wild-type promoter both *in vivo* and *in vitro*, while 15 and 25 bp insertions (about 1.5 and 2.5 helical turns) lowered the activity greatly. Their study indicated that rotational orientation of the curved upstream DNA relative to the Alu156 promoter was of the uppermost importance for efficient promoter function. They did not alter the conformation of the original curvature, while we altered it. However, both studies equally shed light on the importance of the conformation of upstream DNA in efficient transcription.

A similar result was reported by Bracco *et al.* (15). They reported that some synthetic curved DNAs could functionally substitute for the CRP (catabolite activator protein)-induced DNA bend occurring very close to the *gal* promoter. In their study, they also reported that minimal changes in the location of the curved DNA center or perturbation in the magnitude of curvature strongly affected *in vivo* strength of the hybrid *gal* promoter. Collis *et al.* showed that the structural and/or vibrational properties of DNA play a significant role in determining *E. coli* promoter activity (16).

Although the alteration of the curved DNA conformation influenced transcription of the β-lactamase gene *in vivo*, we could not detect any influence on the event *in vitro*. Bracco *et al.* had a similar observation in the study described above (*E. coli* RNA polymerase was used *in vitro*). However, *in vivo* data were corroborated *in vitro* in the studies of McAllister and Achberger (reference 11, they used the major σ^{43} *B. subtilis* RNA polymerase) and Bossi and Smith (reference 7, *E. coli* RNA polymerase was used). Bossi and Smith reported that a 3 bp deletion from the upstream curved DNA sequence of the *his* tRNA promoter of *Salmonella typhimurium* destroyed the curved DNA conformation and reduced transcript level both *in vivo* and *in vitro*. Since the data is limited at present, we can only speculate on the reasons why we and Bracco *et al.* could not reconstitute *in vivo* effects by *in vitro* analyses. In these two cases, for example, some auxiliary factor that recognizes and interacts with the DNA curvature may function in transcription *in vivo*, or as far as our study is concerned, only supercoiled DNA templates with a very specific range of superhelicity may be able to reproduce *in vivo* data *in vitro* (under such a range of superhelicity, a small part of a right-handed coil might be able to stimulate transcription by some unknown means). These possibilities are being examined.

In this study, it was shown that *in vivo* transcription from the β-lactamase promoter is dependent, in part, on the gross geometry of the DNA curvature. In such prokaryotic genes having DNA curvature near the promoter, overall DNA conformation of the region covering the promoter and the curvature seems to play some significant role in efficient transcription. One such role may be related to open complex formation (11, 18, 20, 33). A clue to understanding the role(s) of DNA curvatures in transcription may be obtained by investigating what is (are) lacking in our *in vitro* experiment leading to the discrepancy between *in vitro* and *in vivo* effect on transcription.

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