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Role of Curved DNA in Binding of *Escherichia coli* RNA Polymerase to Promoters

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The ability of curved DNA upstream of the −35 region to affect the interaction of *Escherichia coli* RNA polymerase and promoter DNA was examined through the use of hybrid promoters. These promoters were constructed by substituting the curved DNA from two *Bacillus subtilis* bacteriophage SP82 promoters for the comparable DNA of the bacteriophage λ promoters λp_R and λp_L. The SP82 promoters possessed intrinsic DNA curvature upstream of their −35 regions, as characterized by runs of adenines in phase with the helical repeat. In vitro, the relative affinities of purified σ^{70}-RNA polymerase for the promoters were determined in a competition binding assay. Hybrid promoters derived from λp_R that contained curved DNA were bound by *E. coli* RNA polymerase more efficiently than was the original λp_R. Binding of *E. coli* RNA polymerase to these hybrid promoters was favored on superhelical DNA templates according to gel retardation analysis. Both the supercoiled and relaxed forms of the hybrid λp_L series were better competitors for *E. coli* RNA polymerase binding than was the original λp_L. The results of DNase I footprinting analysis provided evidence for the wrapping of the upstream curved DNA of the hybrid λp_R promoters around the *E. coli* RNA polymerase in a tight, nucleosomal-like fashion. The tight wrapping of the upstream DNA around the polymerase may facilitate the subsequent steps of DNA untwisting and strand separation.

Curved DNA upstream of the −35 region has been implicated in the function of numerous promoters (11, 21, 27). Studies of promoters associated with upstream curved DNA have demonstrated a strong correlation between promoter function and the presence of altered DNA structure, such as sequence-dependent curved DNA (4, 6, 13, 16–18, 21, 26, 27). The presence of curved DNA, as characterized by in-phase adenine tracts, has been found upstream of several promoters from *Escherichia coli*, including the *ompF* promoter (23), the *his* promoter, and the *lpp* promoters (29) and a subset of rRNA promoters (8) and tRNA promoters (5, 6, 16, 18, 25). Deletion analysis of the upstream curved DNA has suggested that promoter activation is intimately related to sequence-dependent curvature (16, 21). In addition, sequence-dependent DNA curvature has been demonstrated to stimulate transcription when curved DNA is used to replace the upstream binding sites for the catabolite activator protein (CAP) (7, 14). This suggests that protein-mediated DNA bending is functionally related to sequence-dependent DNA curvature in transcription initiation.

Some of the most profound effects of curved DNA have been documented in promoters utilized by the *Bacillus subtilis* RNA polymerase. Intrinsically curved DNA upstream of the RNA polymerase binding site strongly influences transcription in *B. subtilis*. Indeed, deletions which eliminate the upstream A tracts have been shown to dramatically reduce expression from several promoters in *B. subtilis* (4, 19, 21). Furthermore, the optimal positioning of the curvature relative to the position of the promoter is essential for efficient promoter utilization in *B. subtilis* (22).

In this report, we examined the ability of curved DNA upstream of the −35 binding site to affect the binding of *E. coli* σ^{70}-RNA polymerase. A series of hybrid promoters in which the curved DNA upstream of the −35 region in the *B. subtilis* bacteriophage SP82 promoters Alu156 and Bal129 was substituted for the analogous region of the lambda promoters λp_R and λp_L were constructed. This substitution yielded hybrid promoters which contained the upstream curved DNA of Alu156 or Bal129 fused to the −10 and −35 sequences of the λp_R and λp_L promoters. With these hybrid promoters, the effect of upstream curved DNA on the binding of *E. coli* RNA polymerase was examined by nitrocellulose filter binding, gel retardation, and DNase I footprinting analysis. Curved DNA facilitates the wrapping of DNA upstream of the −35 region of the promoter around the RNA polymerase.

MATERIALS AND METHODS

Construction of λp_R- and λp_L-derived promoters. DNA restriction fragments containing the λp_R or λp_L promoter were isolated from plasmid pGW7 (10) by digestion with *Alu*I or *Hae*III, respectively. The resulting blunt-ended fragments were purified and ligated into the *Hin*cII restriction site of pUC8 (30) and subsequently were screened by restriction analysis to ensure proper promoter orientation with respect to the restriction sites in the pUC8 vector. The λp_R and λp_L promoters each contain a unique *Hin*cII site, which allows the DNA to be cleaved at position −33 relative to the start of transcription (9). The isolation, purification, and cloning of the bacteriophage SP82 promoters Alu156 and Bal129 into plasmid pUC8 were described previously (21). Each of these promoters can be cut by *Hin*cII at position −33. The presence of an *Hin*cII site located at the same position in each of the promoters facilitated the substitution of the DNA upstream of position −33 in promoters Alu156 and Bal129 for the analogous DNA region in the λp_R and λp_L promoters.

To make the hybrid promoter constructs, the DNA upstream of the Alu156 and Bal129 promoters was purified as *Eco*RI-*Hin*cII restriction fragments from 6% polyacrylamide gels (acrylamide to bisacrylamide, 60:1) as described previously (21). The DNA fragments from the Alu156 and Bal129 promoters were then ligated upstream of the λp_R and λp_L promoters, whose original upstream regions had been deleted by digestion with *Eco*RI and *Hin*cII. This resulted in the formation of hybrid promoters containing the upstream curved elements of the Alu156 or Bal129 promoter and the downstream −10 and −35 sequences of the lambda λp_R or λp_L promoters.

RNA polymerase isolation. RNA polymerase was isolated and purified from *E. coli* MRE600 (ATCC 29417) as described by Achberger and Whiteley (2), except that the enzyme was eluted from DNA cellulose with a gradient of 0.1 to 0.6 M NaCl. Fractions were pooled on the basis of purity and saturation with σ^{70}. The

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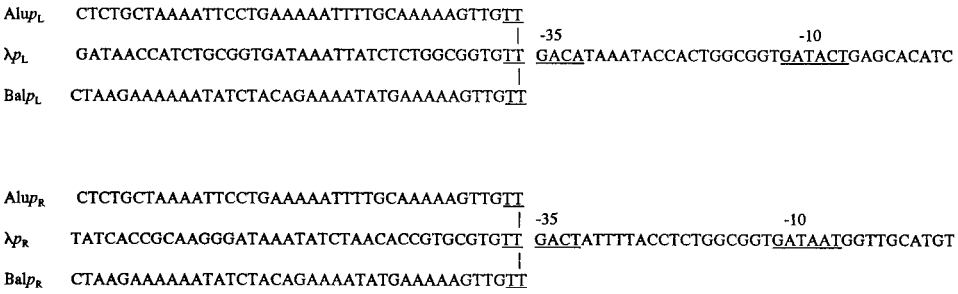

FIG. 1. Nucleotide sequences of the λp_R and λp_L promoters. The nucleotide sequences of the λp_R and λp_L promoters are shown along with the nucleotide sequences of the curved DNAs from the *B. subtilis* phage SP82 promoters Alu156 and Bal129. These curved DNA sequences were used to construct the hybrid promoters in this study. The −10 and −35 regions of each promoter have been underlined.

enzyme was stored at −20°C in 40% glycerol, 10 mM Tris, 1 mM EDTA, and 200 mM NaCl (pH 7.8).

Nitrocellulose filter binding assay. *E. coli* RNA polymerase-dependent retention of promoter-containing DNA fragments on nitrocellulose membrane filters (BA85 membrane; Schleicher & Schuell) was based on the competition binding assay described previously (21). To evaluate the relative affinities of RNA polymerase for the various promoter constructs described above, equal molar amounts of each promoter were used to compete for limiting amounts of RNA polymerase. To generate promoter-containing DNA fragments that could be resolved on a 6% polyacrylamide gel, plasmids containing the promoters were digested with specific combinations of restriction endonucleases. DNA fragments containing the λp_L, Alup_L, Alup_R, or Balp_R promoter were excised from the pUC8 vector by digestion with *Eco*RI and *Pst*I. The λp_R promoter-containing fragment was prepared by digestion with *Eco*RI and *Hin*dIII, while the Balp_L promoter fragment was removed from the vector with *Bam*HI and *Pst*I. Each RNA polymerase binding assay contained 1.5 μg of digested plasmid DNA for each promoter tested. DNA bands were quantified on polyacrylamide gels by ethidium bromide staining and densitometry of photographic negatives. RNA polymerase binding to individual promoter-containing DNA fragments was expressed as a fraction of input DNA.

Gel mobility shift analysis. The relative affinities of *E. coli* RNA polymerase for both linear and supercoiled DNA templates were measured by gel mobility shift analysis (3). DNA restriction fragments containing the λp_R or λp_L promoter were purified as described previously (20) and labeled with T4 polynucleotide kinase and [γ-^{32}P]ATP (Dupont, New England Nuclear). The end-labeled promoter-containing fragments were titrated with *E. coli* RNA polymerase by the filter binding assay to ensure that subsaturating amounts of enzyme were used. *E. coli* RNA polymerase was incubated with approximately 10,000 cpm (0.3 to 0.8 ng) of end-labeled promoter DNA with λp_R or λp_L in the presence of 0, 0.1, 0.25, 0.5, and 1.0 μg of unlabeled specific competitor DNA. Incubations were carried out at 37°C for 5 min in a binding buffer consisting of 40 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, 200 mM NaCl, and 0.6 mM dithiothreitol. In each assay, the nonspecific competitor DNA, pUC18, plus specific competitor DNA equaled 1 μg. Specific competitors were derivatives of pUC8 containing λp_R or λp_L or the hybrid promoters Alup_R, Balp_R, Alup_L, or Balp_L. Protein-DNA complexes were resolved on 4% polyacrylamide gels (acrylamide to bisacrylamide, 79:1) with a high-ionic-strength buffer (3). Shifted DNA fragments were quantified by densitometry of autoradiograms. Competition between the labeled target DNA and specific competitor DNA was presented relative to the amount of DNA shifted in the presence of 1 μg of nonspecific competitor.

DNase I footprint analysis. The sensitivity of RNA polymerase-promoter DNA complexes to DNase I (1) was used to test for the structuring of DNA upstream of the promoter. The promoter-containing fragments λp_R, Alup_R, and Balp_R were labeled at a single 3′ end with [α-^{32}P]dATP and the Klenow fragment of *E. coli* DNA polymerase I and were purified as described previously (20). DNase I footprinting analyses were performed in 180-μl reaction mixtures containing 40 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, 200 mM NaCl, 30,000 cpm of end-labeled, promoter-containing DNA, and *E. coli* RNA polymerase. The reaction mixtures were incubated for 5 min at 37°C prior to the addition of an appropriate dilution of DNase I (Boehringer Mannheim Corp.). After 1 min of nuclease digestion, 19 μl of 3 M sodium acetate and 1 μl of yeast tRNA (1 mg/ml) were added to the reaction mixtures, and this was followed by the addition of 200 μl of phenol to stop the digestions. The aqueous layer containing the DNA was recovered and further purified by ethyl ether extraction and ethanol precipitation.

To minimize the possibility of multiple RNA polymerase molecules binding to a single DNA fragment, the amount of enzyme needed to give 60 to 90% of the maximum binding was determined by the filter binding assay. The DNase I cleavage sites that generated the bands on the autoradiogram were identified by the sequence of purines determined from a G>A chemical sequencing reaction (20) by using the end-labeled promoter-containing DNA fragments. For the DNase I digestions, densitometry of autoradiograms was used to compare the intensities of DNA bands in the control and experimental lanes.

RESULTS

Construction of hybrid promoters. To examine the effect of intrinsic DNA curvature on interactions between *E. coli* RNA polymerase and promoter DNA, we constructed a series of hybrid promoters. Curved DNA located upstream of the −35 region in the Alu156 and Bal129 promoters (21) was substituted for the analogous region of bacteriophage lambda promoters λp_R and λp_L. Hybrid promoter construction was facilitated by the presence of an *Hin*cII restriction site within the −35 region of all the promoters used in this study. The hybrid promoters were designated Balp_R, Balp_L, Alup_R, and Alup_L. For these designations, the Bal- and Alu- prefixes correspond to the presence of the curved DNA from the Bal129 and Alu156 promoters, respectively, and p_R and p_L indicate the presence of DNA downstream of the −35 region from the λp_R and λp_L promoters, respectively.

The nucleotide sequences immediately upstream of the −35 region for the Alu156 and Bal129 promoters are shown in Fig. 1. These promoters possess intrinsic DNA curvature, as characterized by runs of adenines with a 10- to 11-bp periodicity. Although the nucleotide sequences of the curved DNA associated with the Alu156 and Bal129 promoters are similar, they are not identical and would be predicted to have similar but distinct structures. In Fig. 1, the nucleotide sequences for the bacteriophage lambda promoters λp_R and λp_L show that both promoters differ by only one nucleotide from each other in their −10 and −35 regions and have 17-bp spacings between these sites (9). Both of these promoters interact efficiently with the major *E. coli* RNA polymerase, the σ^{70}-containing enzyme (24).

Relative affinities of *E. coli* RNA polymerase for promoters by the filter binding assay. To determine the relative affinities of RNA polymerase for the λ-derived promoters, a series of competition assays were performed by the nitrocellulose filter binding method. In these assays, equal molar amounts of the different promoter-containing DNA fragments competed for binding to RNA polymerase at various concentrations of enzyme. Representative results of the competition binding assay with the λp_R- and λp_L-derived promoter-containing fragments are presented in Fig. 2. The three promoters in the λp_L series displayed no significant differences in binding with RNA polymerase (Fig. 2A). RNA polymerase displayed a greater affinity for the λp_R-derived hybrid promoters, Alup_R and Balp_R, than for the original λp_R promoter (Fig. 2B). The Alup_R promoter was retained on the nitrocellulose filter by RNA polymerase

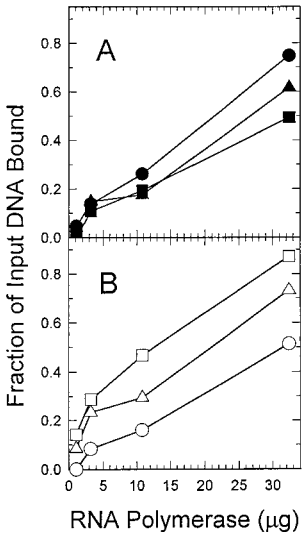

FIG. 2. Relative affinities of RNA polymerase for the promoters λp_R and λp_L and their derivatives. The competition binding assay measured the nitrocellulose filter retention of the promoter-containing DNA fragments as a function of *E. coli* RNA polymerase concentration. At each RNA polymerase concentration, the binding reaction mixture contained equal molar amounts of DNA fragments containing the λp_L (●), Alup_L (■), and Balp_L (▲) promoters (A) and the λp_R (○), Alup_R (□), and Balp_R (△) series of promoters (B).

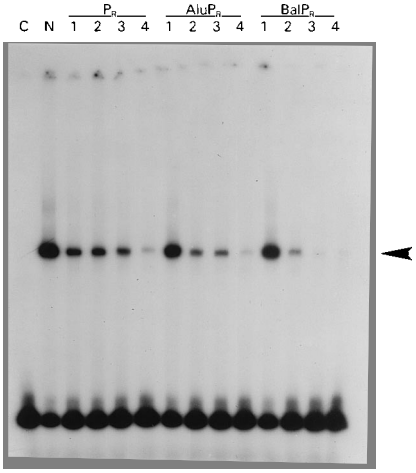

FIG. 3. Use of the gel retardation assay to measure the binding of *E. coli* RNA polymerase to λp_R in the presence of supercoiled DNA competitors. An end-labeled DNA fragment containing the promoter λp_R was added to various amounts of unlabeled supercoiled competitor DNA. The DNA mixture was incubated with 0.75 μg of *E. coli* RNA polymerase at 37°C for 5 min, and this was followed by electrophoresis. All reaction mixtures (except that in lane C) contained *E. coli* RNA polymerase. Lane C is a control lane and represents unbound λp_R. Lane N contains 1 μg of nonspecific competitor DNA (pUC8). Lanes 1, 2, 3, and 4 contain 0.1, 0.25, 0.5, and 1.0 μg, respectively, of specific competitor DNA (plasmid DNA containing λp_R, Alup_R, or Balp_R). The arrow indicates the band observed for the labeled DNA bound by *E. coli* RNA polymerase.

more effectively than any other promoter tested. The addition of curved DNA to the λp_R promoter affected the formation of the RNA polymerase-promoter complexes detected by the nitrocellulose filter binding assay. These complexes were formed with linear DNA fragments and were stable enough to be collected by filtration.

Gel retardation analysis of the relative affinities of RNA polymerase for linear and supercoiled molecules. A series of competition assays to determine the relative affinities of *E. coli* RNA polymerase for hybrid promoters on supercoiled and linearized templates were performed. RNA polymerase binding to end-labeled promoter DNA fragments containing either λp_R or λp_L was measured as a function of the supercoiled or linear competitor DNA concentration by gel retardation analysis.

All the reaction mixtures contained a constant amount of end-labeled DNA fragment containing the λp_R or λp_L promoter. When a specific competitor DNA was used, the amount of nonspecific DNA was decreased so that there was always 1 μg of unlabeled DNA in each assay. The competitions were performed by mixing the designated amounts of unlabeled specific competitor DNA with end-labeled promoter DNA prior to the addition of RNA polymerase. Products were resolved by gel electrophoresis. Fig. 3 depicts a representative autoradiogram from the gel retardation analysis in which the end-labeled promoter DNA was λp_R and all the DNA competitors were negatively supercoiled. In supercoiled form, Balp_R is the most effective of the λp_R series of promoters in competing for binding to RNA polymerase. Supercoiled Alup_R was less effective in binding RNA polymerase than Balp_R but was more effective than the original λp_R promoter. Analogous experiments using competitor DNA relaxed by restriction enzyme digestion were performed. The autoradiograms were quantified by densitometry, and the results were normalized to the intensity of the band shift observed in the absence of specific competitor DNA.

The results for the gel retardation analysis are presented for the λp_L promoter series (Fig. 4) and the λp_R promoter series (Fig. 5). In general, the promoter-containing DNA competitors were much more effective (i.e., bound the RNA polymerase more efficiently) when supercoiled (Fig. 4B and 5B) than when the same plasmid DNAs were relaxed by restriction digestion (Fig. 4A and 5A). In either form, the plasmid DNAs

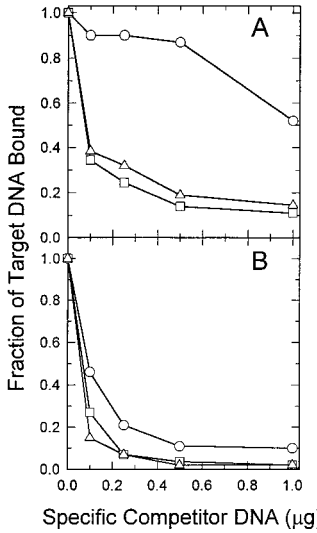

FIG. 4. *E. coli* RNA polymerase binding to λp_L-derived promoters as a function of DNA superhelicity. Gel retardation analysis was used to measure the affinity of *E. coli* RNA polymerase for the λp_L promoter in the presence of various amounts of unlabeled competitor DNA with the λp_L, Alup_L, or Balp_L promoter. Specific competitor DNAs were added in either relaxed (A) or supercoiled (B) form. All reaction mixtures contained a constant amount of end-labeled DNA fragment containing the λp_L promoter. Specific competitor DNAs with λp_L (○), Alup_L (□), and Balp_L (△) promoters were added at the concentrations indicated.

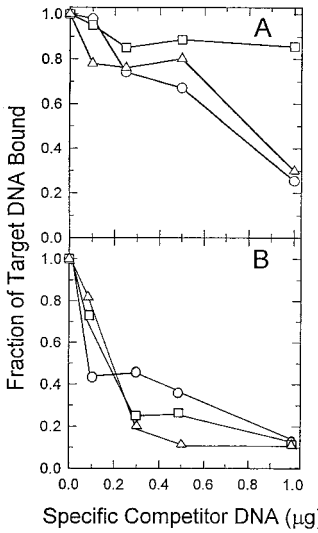

FIG. 5. Effect of DNA superhelicity on *E. coli* RNA polymerase binding to p_R-derived promoters. Gel retardation analysis was used to indirectly estimate the affinity of *E. coli* RNA polymerase for the λp_R series of promoters. In this assay, end-labeled DNA fragments containing the λp_R promoter were incubated with *E. coli* RNA polymerase in the presence of various amounts of unlabeled specific competitor DNA with the λp_R (○), Alup_R (□), or Balp_R (△) promoter. Specific competitor DNAs used in this experiment were either in a relaxed (A) or supercoiled (B) form. Specific competitor DNAs were added at the concentrations indicated.

containing the hybrid promoters derived from λp_L, the Alup_L and Balp_L promoters, were more effective competitors than the original λp_L (Fig. 4). This was most evident when the competitor DNA was relaxed. Since the labeled DNA fragment used in the gel shift assay contained λp_L, all competition was relative to that of the original promoter.

For all competitor DNA containing the λp_R-derived promoters, the supercoiled forms (Fig. 5B) were much more effective in binding RNA polymerase than were the relaxed forms (Fig. 5A). In the relaxed form, the Alup_R promoter failed to significantly compete with the original λp_R promoter target DNA. In the same experiment, the Balp_R hybrid and original λp_R promoters were equally effective. When in the supercoiled form, the hybrid promoters with curved DNA were more efficient competitors than the λp_R promoter. The Balp_R promoter was the most productive competitor. The results of this analysis indicate that on negatively supercoiled DNA templates, curved DNA made a positive contribution to the interactions between *E. coli* RNA polymerase and the promoter.

DNase I footprinting analysis of the interactions of *E. coli* RNA polymerase with the λp_R-derived promoters. Analysis of RNA polymerase binding to the λ-derived promoters provided evidence that the curved DNA upstream of the −35 region contributed to enzyme-promoter interactions. This effect might be due to the direct interaction of the upstream curved region with the polymerase. Thus, DNase I footprinting was used as a method to probe for interactions between upstream regions of DNA curvature and RNA polymerase. Distortion of the DNA helix by DNA-bending proteins may lead to regions of DNase I hypersensitivity, as the phosphodiester bonds are made more accessible to the nuclease.

Fig. 6 shows a representative autoradiogram of the DNase I footprint obtained for RNA polymerase bound to DNA fragments containing λp_R, Alup_R, or Balp_R. A strong protection from DNase I digestion (marked by vertical lines) was observed in the −35 region and the flanking regions of each of the promoters, which is indicative of RNA polymerase-promoter interactions. When the reaction mixture was electrophoresed for shorter periods of time, a DNase I footprint extending to the +15 region was observed. Among the three promoters, the patterns of DNase I-generated bands observed between the −35 region and +15 region were indistinguishable. The extent of this protected region was typical for *E. coli* RNA polymerase bound to other promoters. DNase I-hypersensitive sites (marked by arrows) were indicative of structural alterations of the DNA bound by RNA polymerase. In each of the three promoters, there is a region of enhanced DNase I cleavage centered at −46 which is immediately followed by an additional short region of DNase I protection around −50. Upstream of this point, the patterns obtained for the hybrid promoters with curved DNA deviate from that observed with λp_R. Peak profiles generated by densitometry (Fig. 7) were used to identify regions of helix distortion characterized by DNase I-hypersensitive sites. For the Alup_R promoter, major DNase I-hypersensitive sites were centered at −46, −56, and −66, with significantly less prominent sites at −72, −75, −80, −87, −88, and −90. The Balp_R hybrid promoter bound by RNA polymerase exhibited enhanced DNase I cleavage centered around −46, −56, −68, and −75. The sites that were hypersensitive to DNase I were separated by approximately 10 bp, corresponding to the helical periodicity of the DNA. This pattern of DNase I-hypersensitive sites was observed with the Alup_R and Balp_R hybrid promoters but not with the λp_R promoter.

The location of the sites hypersensitive to DNase I was at or adjacent to the 5′ end of a run of adenines. This location corresponds to the outside of the curve in intrinsically curved DNA. Thus, the DNA was bent by the RNA polymerase in the

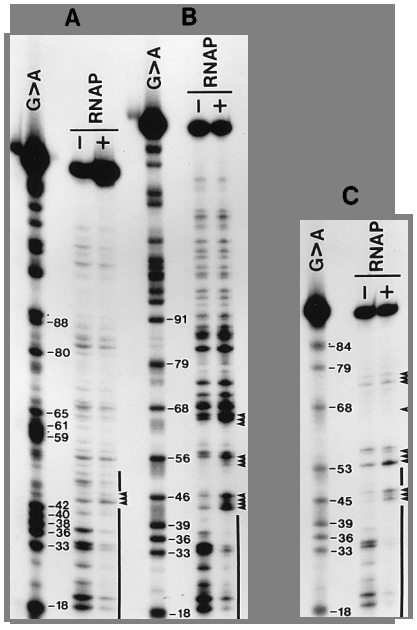

FIG. 6. DNase I footprint analysis of *E. coli* RNA polymerase on λp_R-derived promoter-containing DNA fragments. DNase I footprinting was performed as described in the Materials and Methods section. Shown are footprints of reaction mixtures containing λp_R (A), Alup_R (B), and Balp_R (C). Singly end-labeled DNA fragments containing the promoters λp_R, Alup_R, or Balp_R were incubated in the presence (+) or absence (−) of RNA polymerase. Guanine residues in the G>A sequencing reaction results for each of the λp_R-derived promoters have been numbered for sequence reference with DNase I cleavage patterns. Positions corresponding to DNase I-hypersensitive sites are indicated by arrows. Vertical lines indicate regions of protection from DNase I cleavage.

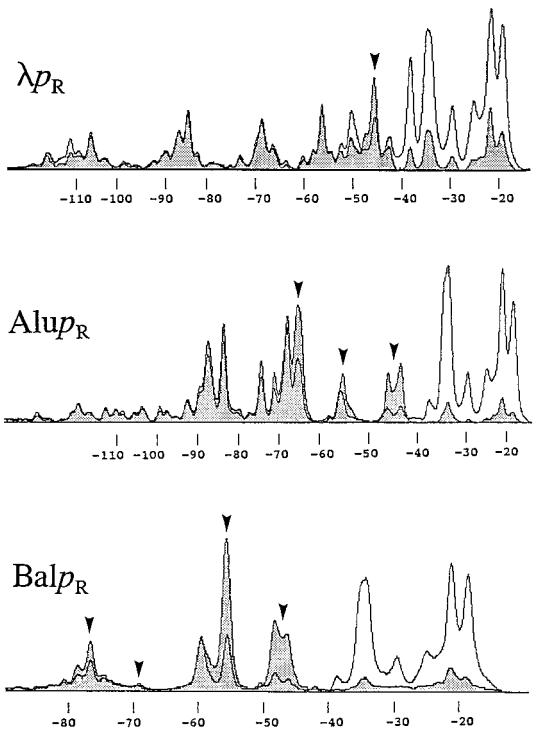

FIG. 7. Densitometry of autoradiograms for DNase I footprinting analysis. Superimposed peak profiles for promoter-containing DNA fragments treated with DNase I in the absence (open tracing) or presence (shaded tracing) of RNA polymerase. DNA protected from DNase I digestion by RNA polymerase is depicted by the open portion of the tracings. The arrows indicate regions of DNA that were hypersensitive to DNase I digestion. The profiles are numbered relative to the transcription initiation site.

same direction as the sequence-dependent curvature. This suggests that the curved DNA of Alup_R and Balp_R is positioning the DNA in such a way as to facilitate its wrapping around the polymerase. The footprint analysis of the λp_R promoter with RNA polymerase did not exhibit the periodic pattern of enhanced cleavage observed with the hybrid p_R promoters.

DISCUSSION

In this communication, we provide evidence that intrinsically curved DNA located upstream of the −35 promoter region is able to affect the interaction of *E. coli* RNA polymerase with both supercoiled and relaxed DNA templates. We used the techniques of nitrocellulose filter binding, gel retardation, and DNase I footprinting to characterize the interactions of *E. coli* RNA polymerase with hybrid promoters containing upstream curved DNA. The hybrid promoters with curved DNA were bound by RNA polymerase more effectively than were the original promoters when the DNA templates were supercoiled, as measured by the gel retardation assay. However, depending on the method used to assay the RNA polymerase-promoter complexes, the interaction of RNA polymerase with hybrid promoters on relaxed DNA templates was promoter specific. By the filter binding assay, curved DNA enhanced the binding of RNA polymerase to the λp_R series of hybrid promoters but had little effect on hybrid promoters in the λp_L series. By the gel retardation assay, in which the competitor DNA molecules were relaxed by restriction enzyme digestion, the λp_R-derived promoters containing curved DNA were not effective competitors while the hybrid promoters in the λp_L series were highly effective. The difference in these results may reflect the inherent differences between the assay techniques. Since the filter binding assay directly measures those complexes stable enough to be detected on a nitrocellulose filter, it would be predicted to miss an effect of curved DNA if it affected the initial binding, the structuring of the DNA, and the formation of relatively weak promoter complexes. However, the gel retardation assay effectively detects even weakly bound protein-DNA complexes which are too unstable to be retained on a nitrocellulose filter. This hypothesis is supported by the recent work of Fried and Liu (12) in which the kinetics of CAP dissociation from the *lac* promoter fragment was examined by gel mobility shift and the filter binding assays. The authors found that the lifetimes of CAP-DNA complexes observed in gels were significantly longer than those of the same complexes in free solution, and they proposed that the stabilization effect observed in gels was due to a reduction of the dissociation rate of the complexes as a result of the molecular sequestration of the reactants.

The ability of curved upstream DNA sequences from Bal129 and Alu156 to enhance the binding affinity for *E. coli* RNA polymerase to promoters on DNA fragments was intriguing. Specifically, results from filter binding analysis indicated that the hybrid promoters Alup_R and Balp_R demonstrated an enhanced binding affinity for *E. coli* RNA polymerase relative to that of the original λp_R promoter. To our knowledge, this is the first demonstration that DNA curvature located immediately upstream of the −35 region affects *E. coli* RNA polymerase binding to promoters on linearized templates. Previous experiments have demonstrated that the replacement of the CAP site on the *lac* and *gal* promoters with curved DNA functionally substituted for CAP-cyclic AMP activation of these promoters, but only with negatively supercoiled templates (7, 14). No stimulation of transcription was observed on DNA fragments. In agreement with the results of these studies, our results from the gel retardation analysis indicated that curved DNA added to the λp_R or λp_L promoter had its most significant effect on RNA polymerase binding when the DNA templates were supercoiled.

Results from the gel retardation and filter binding analyses suggested that *E. coli* RNA polymerase interacts with the λp_R promoter differently than it does with the hybrid promoters possessing DNA curvature upstream of the −35 region. DNase I footprinting was therefore chosen to characterize the interaction between *E. coli* RNA polymerase and the λ-derived promoters. The results of footprinting analysis with the λp_R hybrid promoters suggested that the addition of curvature immediately upstream of the −35 region of the λp_R promoter structured the DNA by wrapping it around the polymerase in a tight, nucleosomal-like fashion. A direct consequence of this structuring would be the formation of additional contacts between the polymerase and the DNA. Although DNA-bending proteins, such as CAP, are known to structure the DNA in an analogous manner upon binding, to our knowledge, the present study provides the first direct evidence for the wrapping of DNA around *E. coli* RNA polymerase without the aid of an additional DNA-bending protein.

It is unclear from the present study whether the RNA polymerase directly binds the curved DNA or the DNA upstream of the curved DNA. However, the presence of alterations in the DNase I pattern upstream of the curved DNA in the hybrid promoters indicates that the RNA polymerase binds this DNA to some extent. If this is true, then the curved DNA would serve, in part, to loop the DNA back to the RNA polymerase. In support of this hypothesis, previous work in our laboratory led to the proposal of a model in which curved DNA upstream of the −35 region of the promoter facilitated the binding of *B.*

subtilis σ^A-RNA polymerase. The curved DNA was proposed to loop the DNA upstream of the promoter back to the RNA polymerase and permit the binding of the enzyme. This model was supported by the finding that A-tract DNA could be moved (i.e., by double-stranded oligonucleotide insertion) 11 and 21 bp away from the promoter without significant loss of promoter function (22). When the curved DNA was moved in increments other than the 10 to 11 bp consistent with the helical repeat of B-form DNA, promoter function was impaired. The rotational orientation and not the linear displacement of the curved DNA was the most critical factor in stimulating promoter binding by the *B. subtilis* RNA polymerase.

Another model to explain the enhanced promoter function observed when DNA rich in A+T is located upstream of the −35 region has been proposed. Using the *E. coli rrnB* P1 promoter, Ross et al. (28) provided direct evidence for the binding of the RNA polymerase α subunit to the DNA rich in A+T. This binding accompanied a 30-fold increase in transcription from this promoter. These authors go on to suggest that promoters that have DNA rich in A+T, including those with A tracts, may also interact with the α subunit. The A+T-rich region of the *rrnB* P1 promoter does not have the sequence indicative of curved DNA.

In our study, we find a short region of protection from DNase I around the −50 position on each of the promoters in the λp_R series. This is the same general area characterized by DNase I footprinting as the A+T-rich α subunit binding site on the *rrnB* P1 promoter (28). Each of the λp_R-derived hybrid promoters has an A tract at this position, and the original λp_R has a 12-bp A+T sequence at this site. While we cannot preclude a role for direct binding of the RNA polymerase to this site, we have evidence for much more extensive interactions upstream of this site in the presence of curved DNA. There was no evidence of DNA structuring via wrapping in the work with the *rrnB* P1 promoter (28) or in our work with the λp_R promoter. The extensive wrapping observed for promoters with curved DNA is sufficient to negate the suggestion by Ross et al. (28) that enhanced RNA polymerase binding ascribed to curved DNA was attributable to the same α subunit binding effect observed for the *rrnB* P1 promoter.

DNA wrapping or structuring may well be a mandatory step in transcription initiation at all promoters recognized by the major *E. coli* RNA polymerase. In our experiments, the addition of curved DNA to the λp_R promoter facilitated DNA wrapping and placed most of the RNA polymerase-promoter complexes in the structured state. This allowed detection of the structured complex by DNase I footprinting. In the absence of DNA curvature, a small proportion of the RNA polymerase-promoter complexes may exist in the structured state at any one time, making detection difficult. This study provides direct evidence in support of the models citing the contribution of DNA structuring by RNA polymerase to the initiation of transcription.

REFERENCES

1. **Achberger, E. C., M. D. Hilton, and H. R. Whiteley.** 1982. The effect of the delta subunit on the interaction of *Bacillus subtilis* RNA polymerase with bases in a SP82 early gene promoter. Nucleic Acids Res. **10:**2893–2910.
2. **Achberger, E. C., and H. R. Whiteley.** 1980. The interaction of *Escherichia coli* core RNA polymerase with the specificity-determining subunits derived from unmodified and SP82 modified *Bacillus subtilis* RNA polymerase. J. Biol. Chem. **255:**11957–11964.
3. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl.** 1989. Current protocols in molecular biology. Greene Publishing Associates & Wiley Interscience, New York.
4. **Banner, C. D. B., C. P. Moran, and R. Losick.** 1983. Deletion analysis of a complex promoter for a developmentally regulated gene from *Bacillus subtilis*. J. Mol. Biol. **168:**351–365.
5. **Bossi, L.** 1983. The *hisR* locus of *Salmonella*: nucleotide sequence and expression. Mol. Gen. Genet. **192:**163–170.
6. **Bossi, L., and D. M. Smith.** 1984. Conformational change in the DNA associated with an unusual promoter mutation in a tRNA operon of *Salmonella*. Cell **39:**643–652.
7. **Bracco, L., T. Kortlarz, A. Kolb, S. Diekmann, and H. Buc.** 1989. Synthetic curved DNA sequences can act as transcriptional activators in *Escherichia coli*. EMBO J. **8:**4289–4296.
8. **Brosius, S. S., T. J. Dull, D. D. Sleeter, and H. F. Noller.** 1981. Gene organization and primary structure of a ribosomal RNA operon from *Escherichia coli*. J. Mol. Biol. **148:**107–127.
9. **Daniels, D. L., J. L. Schroeder, W. Szybalski, F. Sanger, A. R. Coulson, G. F. Hong, D. F. Hill, G. B. Petersen, and F. R. Blattner.** 1983. Complete annotated lambda sequence, p. 519–661. *In* R. W. Hendrix, J. W. Roberts, F. W. Stahl, and R. A. Weisberg (ed.), Lambda II. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
10. **Deutch, A. H., C. J. Smith, K. E. Rushlow, and P. J. Kretschmer.** 1982. *Escherichia coli* delta-1 pyrroline-5-carboxylate reductase: gene sequence, protein overproduction, and purification. Nucleic Acids Res. **10:**7701–7714.
11. **Espinosa-Urgel, M., and A. Tormo.** 1993. σ^S-dependent promoters in *Escherichia coli* are located in DNA regions with intrinsic curvature. Nucleic Acids Res. **21:**3667–3670.
12. **Fried, M. G., and G. Liu.** 1994. Molecular sequestration stabilizes CAP-DNA complexes during polyacrylamide gel electrophoresis. Nucleic Acids Res. **22:**5054–5059.
13. **Galas, D. J., M. Eggart, and M. S. Waterman.** 1985. Rigorous pattern-recognition methods for DNA sequences: analysis of promoter sequence from *Escherichia coli*. J. Mol. Biol. **186:**117–128.
14. **Gartenberg, M. R., and D. M. Crothers.** 1991. Synthetic DNA bending sequences increase the rate of *in vitro* transcription initiation at the *Escherichia coli lac* promoter. J. Mol. Biol. **219:**217–230.
15. **Giladi, H., M. Gottesman, and A. B. Oppenheim.** 1990. Integration host factor stimulates the phage lambda p_L promoter. J. Mol. Biol. **213:**109–121.
16. **Hsu, L. M., J. K. Giannini, T.-W. C. Leung, and J. C. Crosthwaite.** 1991. Upstream sequence activation of *Escherichia coli argT* promoter *in vivo* and *in vitro*. Biochemistry **30:**813–822.
17. **Kuhnke, G., H. J. Fritz, and R. Ehring.** 1987. Unusual properties of promoter-up mutations in the *Escherichia coli* galactose operon and evidence suggesting RNA polymerase-induced DNA bending. EMBO J. **6:**507–513.
18. **Lamond, A. I., and A. A. Travers.** 1983. Requirement for an upstream element for optimal transcription of a bacterial tRNA gene. Nature (London) **305:**248–250.
19. **Lee, G., C. Talkington, and J. Pero.** 1980. Nucleotide sequence of a promoter recognized by *Bacillus subtilis* RNA polymerase. Mol. Gen. Genet. **180:**57–65.
20. **Maxam, A., and W. Gilbert.** 1980. Sequencing end-labeled DNA with base-specific chemical cleavages. Methods Enzymol. **65:**499–560.
21. **McAllister, C. F., and E. C. Achberger.** 1988. Effect of polyadenine-containing curved DNA on promoter utilization in *B. subtilis*. J. Biol. Chem. **263:**11743–11748.
22. **McAllister, C. F., and E. C. Achberger.** 1989. Rotational orientation of upstream curved DNA affects promoter function in *B. subtilis*. J. Biol. Chem. **264:**10451–10456.
23. **Mizuno, T.** 1987. Static bend of DNA helix at the activator recognition site of the *ompE* promoter in *Escherichia coli*. Gene **54:**57–64.
24. **Mulligan, M., D. Hawley, R. Entriken, and W. McClure.** 1984. Promoter sequences predict *in vitro* RNA polymerase selectivity. Nucleic Acids Res. **12:**789–800.
25. **Nishi, T., and S. Itoh.** 1986. Enhancement of transcriptional activity of the *Escherichia coli trp* promoter by upstream A+T rich regions. Gene **44:**29–36.
26. **Pérez-Martín, J., F. Rojo, and V. de Lorenzo.** 1994. Promoters responsive to DNA bending: a common theme in prokaryotic gene expression. Microbiol. Rev. **58:**268–290.
27. **Plaskon, R. R., and R. M. Wartell.** 1987. Sequence distributions associated with DNA curvature are found upstream of strong *E. coli* promoters. Nucleic Acids Res. **15:**785–796.
28. **Ross, W., K. K. Gosink, J. Salomon, K. Igarashi, C. Zou, A. Ishihama, K. Severinov, and R. Gourse.** 1993. A third recognition element in bacterial promoters: DNA binding by the α subunit of RNA polymerase. Science **262:**1407–1413.
29. **Verde, P., R. Frunzio, P. P. di Nocera, F. Blasi, and C. P. Bruni.** 1981. Identification, nucleotide sequence and expression of the regulatory region of the histidine operon of *Escherichia coli* K-12. Nucleic Acids Res. **9:**2075–2086.
30. **Vieira, J., and J. Messing.** 1982. The pUC plasmids, an M13mp7-derived system for insertion mutagenesis and sequencing with synthetic universal primers. Gene **19:**259–268.