Effect of Polyadenine-containing Curved DNA on Promoter Utilization in Bacillus subtilis*

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The effect of DNA upstream of the −35 region on promoter function was examined using two promoters isolated from the Bacillus subtilis bacteriophage SP82. The affinity of RNA polymerase for the two promoters in vitro differed significantly. For each promoter the nucleotide sequence of the upstream DNA was characterized by the presence of successive runs of adenines with a 10–11-base pair periodicity. DNA fragments with the polyadenine-containing upstream DNA displayed aberrant electrophoretic mobilities when analyzed by polyacrylamide gels indicative of curved DNA. A series of mutant promoters in which the upstream DNA was deleted could be partially restored if the template was negatively supercoiled. Measurements of chloramphenicol acetyltransferase specific activity from B. subtilis strains carrying transcriptional fusions indicate that the curved upstream DNA stimulated transcription from the promoter with the weaker affinity for RNA polymerase. The curved DNA reduced the in vivo activity of the promoter with the strong affinity for RNA polymerase. One function of the curved upstream DNA may be to provide RNA polymerase-promoter interactions that facilitate open complex formation.

The major vegetative RNA polymerase from Bacillus subtilis, the σ^70 containing enzyme, recognizes promoters that have a consensus nucleotide sequence very similar to that derived from promoters of Escherichia coli (1–4). In addition to the regions of conserved nucleotide sequence at −10 and −35, promoters efficiently utilized in B. subtilis possess conserved nucleotide sequence at −16 and −43 (1, 4). The sequence centered around −43 is characterized by a short run of adenines.

DNA upstream of the −35 region has been demonstrated to be necessary for a high rate of transcription from some promoters. These promoters include the λ P, promoter (5), the E. coli rnrB P, promoter (6), and the promoters for the Salmonella typhimurium hisR (7) and E. coli tyrT (8) genes. DNA fragments containing two of these promoters, the hisR gene and rnrB P, promoters, display abnormal electrophoretic mobilities often associated with regions of curved DNA (9–12). For these two E. coli promoters, mutations in the upstream regions that reduced promoter function imparted normal electrophoretic mobility to the DNA fragments containing these promoters (6, 7). The correlation between promoters displaying a high rate of transcription initiation and regions of altered DNA conformation has also been made using nucleotide sequence analyses (13, 14).

DNA conformation has also been implicated in the activation of transcription by ancillary proteins. The CAMP-cAMP receptor protein complex appears to induce a bend in the DNA at −66 relative to the lactose operon promoter P1 as part of the activation process (15). Protein-mediated bending or looping of the DNA upstream of promoters has also been proposed for the activation of the E. coli glnAp2 promoter by the glnG (ntrC) gene product (16) and the activation of the Klebsiella pneumoniae nifH promoter by the nifA gene product (17). It may be that this type of DNA bending is functionally analogous to a sequence dependent curve in the DNA upstream of a promoter.

The DNA upstream of promoters in B. subtilis can influence transcription. The B. subtilis spoVG promoter is recognized by minor forms of the RNA polymerase, the σ^32- and σ^31-containing enzymes (18). The utilization of the spoVG promoter by each of these forms of the RNA polymerase was dependent on the upstream DNA.

In this report we examine the importance of DNA upstream of the −35 region to the function of two bacteriophage SP82 promoters recognized by the major B. subtilis RNA polymerase. A series of mutants were constructed from these two promoters in which the upstream DNA was either deleted or replaced with other DNA. Using these mutant promoters, the influence of the upstream DNA on the binding of RNA polymerase and subsequent transcription was examined. The upstream DNA enhanced the binding of RNA polymerase to the downstream promoter sequences and affected the level of transcription both in vitro and in vivo. The electrophoretic analysis of DNA fragments containing these promoters was consistent with the existence of a region of curved DNA immediately upstream of the −35 region.

**EXPERIMENTAL PROCEDURES**

**RESULTS**

To study how transcription initiation by the major RNA polymerase from B. subtilis is influenced by the DNA upstream from the enzyme-binding site, a series of mutant

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1 Portions of this paper (including "Experimental Procedures" and Fig. 2) are presented in miniprint at the end of this paper. The abbreviations used are: CAT, chloramphenicol acetyltransferase; CRP, cAMP receptor protein; bp, base pairs; RF, replicative form; TBE, Tris-borate-EDTA. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are included in the microfilm edition of the Journal that is available from Waverly Press.
promoters were constructed in vitro. The nucleotide sequence of
the two *B. subtilis* bacteriophage SP82 promoters used to
make the mutant promoters is presented in Fig. 1. These
promoters are recognized by the major *B. subtilis* RNA polym-
erase, the α*+-*-containing enzyme. This RNA polymerase rec-
ognizes the same consensus sequence as the major *E. coli*
RNA polymerase. When the promoters used in this study
were compared to the consensus sequence for *E. coli* promoters
using the parameters of Mulligan et al. (36), the Alu156
promoter was 83% homologous and the Ball29 promoter was
90% homologous. For each promoter the nucleotide sequence
upstream from the −35 region contains runs of adenines with a
10–11 base pair periodicity. In addition, the Alu156 and Ball
29 promoters have identical sequence from −31 through −44.
Within this region each promoter has a unique HinCl restriction
enzyme site that was used to construct three classes of
mutants. Hybrid promoters were formed by exchanging the
DNA upstream from the HinCl site between Alu156 and
Ball29. The nucleotide sequences of the resulting promoters,
Alu156 hybrid and Ball29 hybrid, were altered from the original promoters upstream from −44.

Promoters grouped in the second class of mutants each
possessed a deletion of all promoter DNA upstream from −33
and were referred to as the −34 deletions. In these mutants
the thymine-adenine base pairs at −34 and −35 were replaced
by cytosine-guanine base pairs from the vector DNA resulting
in an alteration of the −35 region. The next class of mutants
were constructed in such a way as to remove all promoter DNA
upstream from −35. These mutants, designated the −36
deletions, retained the same −35 region as the original pro-
motors. A variation on the −36 deletion series of mutants
were the Alu156-extended and Ball29-extended promoters in
which an 91-base pair DNA fragment, chosen for its lack of
promoter-related DNA, was added to the upstream terminus of
the −36 deletion mutant. All mutants within a series (i.e.
the Alu156 series or the Ball29 series) have the same DNA
sequence, displayed only 38% of the chloramphenicol acetyl-
transferase specific activity of the original Ball29 promoter.
Deletion of all promoter DNA upstream from −35 from
Ball29 (i.e. the Ball29 −36 deletion) resulted in increased
chloramphenicol acetyltransferase specific activity. The
Ball29 −34 deletion retained 92% of the activity of the original
Ball29 promoter despite containing an altered −35 region as well as the deletion of upstream DNA sequences.

The deletion of upstream DNA sequences had an opposite
effect on the Ball29 and Alu156 promoters. To resolve the
apparent discrepancy, the in vitro interaction of RNA polym-
erase with the promoter constructs was examined.

**RNA Polymerase-Promoter Interactions in Vitro**—To test
if the low in vivo promoter activity of Ball29 and Ball29
hybrid relative to the Alu156 promoter reflects weak interac-
tions with the RNA polymerase, the binding of *B. subtilis*
RNA polymerase to the different promoter constructs was
examined. In the initial series of experiments, equal molar
amounts of the different promoter-containing DNA fragments
competed for subsaturating levels of RNA polymerase. Rep-
resentative results of the competition binding among Ball29,
Ball29 hybrid, Alu156, and Alu156 hybrid are presented in
Fig. 3. Despite the similarities in overall promoter sequence,
the RNA polymerase had a greater affinity for the Ball29
promoters than the Alu156 promoters. No significant binding
to the Alu156 promoters was observed until the Ball29 pro-
motors were nearly saturated by RNA polymerase. The low
in vivo activity of Ball29 and Ball29 hybrid does not appear
to be due to weak binding of RNA polymerase. In addition
when the binding of RNA polymerase was measured as a
function of NaCl concentration in the reaction, the Ball29
promoters were bound more efficiently at higher salt concen-
trations (i.e. 50% of the maximal binding observed at 0.05 M
NaCl) was still present at 0.25 M NaCl for the Ball29 pro-
motors while binding to the Alu156 promoters dropped to
the 50% level at 0.1 M NaCl.

![Fig. 1. Nucleotide sequence of promoter-containing DNA fragments. Bases representing the +1 site, the −35 region, and the −10 region of the Alu156 and Ball29 promoters are in bold type. The runs of adenines in the DNA upstream of the −35 region are underlined.](image-url)
transcriptional fusions. The values were corrected for background.

pendent transformants containing the correct pPL703-promoter
measurements with pPL703 containing
pmol of chloramphenicol acetylated/min/mg of protein at 25 °C.

DNA fragments containing the Ball29 promoter sequence (Fig.
DNA fragments bearing the Alu156 deletion mutant pro-
constructs containing the hybrid promoters. In each case DNA fragments containing promoter
concentration. At each RNA polymerase concentration, the reaction
was quantitated as described under "Experimental Procedures" (Miniprint section) was used to
calculate the relative affinity of RNA polymerase for DNA fragments containing promoters with altered upstream DNA. The competition binding assay was used with DNA fragments carrying the promoters from the Ball29 series (A) or the Alu156 series (B).

The Alu156 promoter DNA fragments bound RNA polymerase more efficiently than those with the Alu156 hybrid promoter sequence (Fig. 4B). RNA polymerase binding to DNA fragments bearing the Alu156 deletion mutant promoters paralleled that observed for the Ball29 series. Even at relatively high concentrations of RNA polymerase, DNA fragments with promoter sequences deleted of upstream regions were inefficiently bound by enzyme.

The rate of dissociation of RNA polymerase from each of the promoter constructs was measured to generate an estimate of RNA polymerase-promoter complex stability. The half-life for RNA polymerase dissociation from Ball29 hybrid promoter-containing DNA fragments was consistently longer than that for Ball29 (Table II). The removal of upstream DNA from the Ball29 promoter resulted in a greater than 10-fold reduction in RNA polymerase-promoter complex stability. RNA polymerase dissociated from complexes with the Alu156 series of promoters with half-lives on the order of 1

FIG. 3. Relative affinity of B. subtilis RNA polymerase for Ball29 and Alu156 promoters. The competition binding assay measured the fraction of promoter-containing DNA retained on a nitrocellulose membrane filter as a function of RNA polymerase concentration. At each RNA polymerase concentration, the reaction contained equal amounts of DNA fragments containing the Ball29 promoter (O), the Ball29 hybrid promoter (A), the Alu156 promoter (O), and the Alu156 hybrid promoter (A). RNA polymerase binding was quantitated as described under "Experimental Procedures" (Miniprint).

The in vitro competition binding assay described under "Experimental Procedures" (Miniprint section) was used to compare the affinity of RNA polymerase for the promoter constructs bearing deletions with the original and hybrid promoters. In each case DNA fragments containing promoter constructs with the intact upstream DNA region were more efficiently bound by the RNA polymerase than those in which the upstream DNA region had been deleted (Fig. 4). In examining the binding of RNA polymerase to promoter DNA fragments, no appreciable binding to promoters lacking upstream DNA regions was apparent until after promoters with intact upstream regions were nearly saturated by enzyme. The additional DNA on the Ball29-extended promoter DNA fragment marginally improved RNA polymerase binding over that observed for the Ball29 −36 deletion promoter used to construct it (Fig. 4A).

The Alu156 promoter DNA fragments bound RNA polymerase more efficiently than those with the Alu156 hybrid promoter sequence (Fig. 4B). RNA polymerase binding to DNA fragments bearing the Alu156 deletion mutant promoters paralleled that observed for the Ball29 series. Even at relatively high concentrations of RNA polymerase, DNA fragments with promoter sequences deleted of upstream regions were inefficiently bound by enzyme.

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<tr>
<td>Alu156</td>
<td>1.2</td>
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<tr>
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<tr>
<td>Alu156 −36 deletion</td>
<td>1.4</td>
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<tr>
<td>Alu156 −34 deletion</td>
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<tr>
<td>Alu156 extended</td>
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<tr>
<td>Ball29</td>
<td>52.8</td>
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<tr>
<td>Ball29 hybrid</td>
<td>59.4</td>
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<td>Ball29 −34 deletion</td>
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The relative affinity of RNA polymerase for DNA fragments containing promoters with altered upstream DNA. The competition binding assay was used with DNA fragments carrying the promoters from the Ball29 series (A) or the Alu156 series (B). At each RNA polymerase concentration tested, the reactions contained equal amounts of DNA fragments with the original promoter (O) and four promoters with altered upstream DNA. Within each series promoter-containing DNA fragments included the hybrid promoter (A), the extended promoter (O), the −36 deletion promoter (D), and the −34 deletion promoter (O). Quantitation of individual DNA fragments was described under "Experimental Procedures" (Miniprint).

FIG. 4. Relative affinity of RNA polymerase for DNA fragments containing promoters with altered upstream DNA. The competition binding assay was used with DNA fragments carrying the promoters from the Ball29 series (A) or the Alu156 series (B). At each RNA polymerase concentration tested, the reactions contained equal amounts of DNA fragments with the original promoter (O) and four promoters with altered upstream DNA. Within each series promoter-containing DNA fragments included the hybrid promoter (A), the extended promoter (O), the −36 deletion promoter (D), and the −34 deletion promoter (O). Quantitation of individual DNA fragments was described under "Experimental Procedures" (Miniprint).

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min. Estimates of RNA polymerase binding and RNA polymerase-promoter complex stability for the Ball129 and Alu156 promoters suggest that the RNA polymerase binds the Ball129 promoters more tightly than the Alu156 promoters. In general there was an inverse correlation between the stability of RNA polymerase-promoter complexes and in vivo activity for the Ball129 series.

Effect of Negative Supercoiling of Template DNA on In Vitro Transcription of Promoter Constructs—DNA bearing each of the promoter constructs was inserted into the plasmid vector pUC8 in the same orientation. Purified plasmid DNA of each clone was used as template for in vitro transcription. The plasmid templates were utilized either in their natural negative supercoiled state or in a form linearized by the restriction enzyme ScaI. E. coli pUC8 DNA is a very poor template for purified B. subtilis RNA polymerase which contains saturating levels of the δ subunit. Typically, the level of transcription from pUC8 DNA was less than 20% of that observed with the least efficient promoter construct used in this study, the Alu156 –34 deletion. The results presented in Fig. 5 were corrected for transcription from pUC8 for each RNA polymerase concentration.

The ability of the different Ball129 promoter constructs to direct transcription from a linear template as a function of RNA polymerase concentration is demonstrated in Fig. 5A. There was a direct correlation between RNA polymerase binding to a promoter construct in the competition binding assay and the level of RNA synthesis measured in the in vitro transcription assay. The best template contained the Ball129 hybrid promoter. The Ball129 and Ball129-extended promoters were also effective in vitro. The Ball129 –36 deletion template gave about two-thirds the level of transcription as the Ball129 promoter. The poorest template in this series of promoters was the Ball129 –34 deletion promoter which demonstrated less than 15% of the Ball129 activity at the greatest RNA polymerase concentration tested. When the same plasmid templates were used in a negatively supercoiled form (Fig. 5C), similar results were observed with two exceptions. The Ball129 hybrid promoter template was less effective relative to the Ball129 promoter in this form. The level of transcription from the Ball129 –34 deletion promoter was elevated 4-fold relative to the other promoters.

B. subtilis RNA polymerase effectively utilized the Alu156 and Alu156 hybrid promoters when present on a linear DNA fragment (Fig. 5B). None of the linear DNA fragments containing the Alu156 mutant promoters lacking the upstream DNA region served as productive templates. The Alu156 –36 deletion promoter allowed less than one-tenth the transcription as Alu156. These results are consistent with the measurements of RNA polymerase binding and in vivo promoter function.

When the Alu156 promoter series were tested for their ability to promote transcription from a negatively supercoiled template, there was a dramatic increase in the level of transcription from promoter constructions missing the upstream DNA region relative to linear templates (Fig. 5D). While Alu156 and Alu156 hybrid promoters were relatively unaffected by the DNA conformational change, there was conservatively a 6–10-fold increase in promoter activity for promoter lacking the upstream DNA region. In this in vitro assay, the negative supercoiling of the template was able to compensate, in part, for the absence of the upstream sequences.

**Electrophoretic Mobilities of Promoter-containing DNA Fragments**—DNA fragments containing either the Alu156 or Ball129 promoters were ligated with pBR322 digests with the restriction enzyme EcoRI. Purified pBR322 and plasmids possessing each of the promoter DNA fragments were linearized with one of a series of restriction enzymes. The linear DNA molecules were electrophoresed through polyacrylamide gels as described under "Experimental Procedures." The apparent molecular weight of each band was estimated using λ DNA digested with HindIII as molecular weight markers. The data in Table IIIA are presented as the ratio of apparent molecular weight determined by polyacrylamide gel electrophoresis to the actual molecular weight derived from the DNA sequence. The insertion of the promoter-containing DNA

**Table III**

<table>
<thead>
<tr>
<th>Apparent molecular weight of promoter-containing DNA fragments</th>
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<tr>
<td>DNA fragments</td>
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<tr>
<td></td>
</tr>
<tr>
<td>A. Linear plasmid DNA</td>
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<tr>
<td>pBR322-PerI</td>
</tr>
<tr>
<td>pBR322-SalI</td>
</tr>
<tr>
<td>pBR322/Alu156-PerI</td>
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<tr>
<td>pBR322/Alu156-SalI</td>
</tr>
<tr>
<td>pBR322/Ball129-PerI</td>
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<tr>
<td>pBR322/Ball129-SalI</td>
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<tr>
<td>B. DNA fragments</td>
</tr>
<tr>
<td>Alu156</td>
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<tr>
<td>Alu156 –36 deletion</td>
</tr>
<tr>
<td>Alu156 –34 deletion</td>
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<tr>
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<td>Ball129 extended</td>
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fragments into pBR322 resulted in the altered electrophoretic mobility of the linear DNA. The Alu156 promoter-containing plasmid digested with *Pst*I displayed an apparent molecular weight 43% greater than the actual molecular weight. When the Bal129 promoter plasmid was linearized with the same enzyme, its apparent molecular weight was 40% greater than the actual molecular weight. Similar observations were made when the plasmids were digested with *Sal*I. The apparent molecular weight of pBR322 was only 3% greater than the actual molecular weight under the same conditions. The small changes in apparent molecular weight of pBR322 were consistent with the work of Stellwagen (37).

Estimates of molecular weight based on the electrophoretic mobilities of small, promoter-containing DNA fragments in polyacrylamide also deviated from the actual molecular size (Table IIII). The Alu156 and Bal129 promoter DNA fragments routinely migrated at a rate consistent with a DNA molecule 20–22% larger than their actual size. Promoter constructs in which the polyadenine-containing upstream DNA sequences had been deleted migrated less abnormally. The discrepancy between apparent and actual molecular weight was further reduced by the replacement of upstream DNA sequences with nonpromoter DNA in the Alu156-extended and Bal129-extended promoters. The abnormally slow polyacrylamide gel mobility of the Alu156 and Bal129 promoter DNA fragments may be indicative of altered DNA conformation. DNA sequences responsible for the altered mobility are contained, in part, in the DNA upstream from the −35 region.

**DISCUSSION**

Despite similarities in the nucleotide sequences of the Alu156 and Bal129 promoters (i.e. they share 67% homology between nucleotides −44 and +1, Fig. 1), the deletion of DNA immediately upstream from the −35 region affected each promoter differently. For this reason the results obtained with each promoter will, initially, be discussed separately.

The Alu156 Promoter—In general efficient utilization of the Alu156 promoter was dependent on the DNA upstream from the −35 region. When the upstream DNA from the Bal129 promoter was substituted for the analogous DNA in the Alu156 promoter, the hybrid retained two-thirds of the original activity in *vivo*. However, the deletion of upstream DNA from the Alu156 promoter resulted in a 10-fold decrease in promoter function. In addition relative to the original promoters, DNA fragments with the upstream deletion displayed reduced binding by RNA polymerase in competition binding assays. These results indicated that the formation of RNA polymerase-promoter complexes was influenced by the DNA upstream from the −35 region.

The results of *in vitro* transcription from linear DNA templates mimicked those obtained in *vivo*; there was a 10-fold decrease in transcription when the upstream DNA was deleted. The loss of promoter function for Alu156 promoters lacking the upstream DNA was partially restored if the DNA templates were negatively supercoiled. The mutants Alu156 −36 deletion and Alu156 extended, which differed in nucleotide sequence upstream from −41, displayed elevated transcription from supercoiled templates indicating that this effect was not a function of the sequence used to replace the polyadenine-containing upstream DNA. It is possible that the untwisting due to the negative supercoiling of these templates directly compensated for the loss of the upstream DNA. The untwisting of the DNA helix is a mandatory step in the formation of the open promoter complex. Since plasmids isolated using CaCl₂ density gradients may be more untwisted than those found in *vivo* (38), it is difficult to relate the *in vitro* transcription assays from supercoiled templates to *in vivo* chloramphenicol acetyltransferase expression without further investigation.

The Bal129 Promoter—The Bal129 promoter shares the elements of conserved DNA sequence with the consensus promoter sequences derived for gram-positive bacteria (1) and for the major *E. coli* RNA polymerase (2, 39–41). Despite this high homology with the consensus sequences, this promoter displayed 2-fold lower in *vivo* activity than the Alu156 promoter. The deletion of upstream DNA from the Bal129 promoter actually increased in *vivo* promoter function. The upstream DNA essential for the efficient function of the Alu156 promoter reduced Bal129 promoter activity in *vivo*.

Based on RNA polymerase binding assays, the Bal129 promoter has a much greater affinity for RNA polymerase than the Alu156 promoter. In dissociation experiments the half-life of the Bal129 promoter was at least 40-fold longer than the Alu156 promoter. It is possible that the binding between the RNA polymerase and the Bal129 or Bal129 hybrid promoter is so tight that the clearance of the RNA polymerase from the promoter after initiation is impaired. By this reasoning a mutation that decreased the affinity of the RNA polymerase for this promoter could be expected to have little effect or actually enhance in *vivo* promoter function (e.g. if the increase in promoter clearance was more significant than the decrease in promoter binding). Results similar to this were obtained. The Bal129 −36 deletion promoter displayed half again the level of activity as the Bal129 promoter.

The competition binding assay confirmed that, similar to Alu156, the loss of the upstream DNA from the Bal129 promoter decreased the affinity of RNA polymerase for this site. Dissociation studies demonstrated half-lives for Bal129 promoters lacking upstream DNA on the order of one-tenth those determined for the Bal129 and Bal129 hybrid promoters. In fact the Bal129 hybrid promoter, which allowed only low level expression in *vivo*, consistently displayed the longest half-life in dissociation studies. This supports the idea that the low *in vivo* activities of the Bal129 and Bal129 hybrid promoters are due to a reduced ability of the RNA polymerase to leave the promoter following initiation and that the upstream DNA contributes to this tight binding.

Altered Conformation of Promoter DNA—The electrophoretic mobilities of linear DNA fragments containing the Ball129 or Alu156 promoter were examined. The 186-base pair Alu156- and 162-base pair Bal129-containing DNA fragments migrated at a size about 20% larger than predicted. When small DNA fragments containing these promoters were inserted in pBR322, the apparent molecular weight of the entire molecule increased 40% over the actual size. The ability of these small DNA fragments to impart aberrant electrophoretic mobilities on larger DNA molecules and the presence of runs of adenines with a 10.5-base pair periodicity in the DNA immediately upstream from the −35 region are consistent with the existence of a region of curved DNA (10–12). Based on sequence predictions involving the length of the runs of adenines and the spacing that separates them, the upstream region from Alu156 would possess greater sequence-dependent curvature than that from Bal129.

Several models have been proposed to explain the stimulation of transcription by DNA upstream of the −35 region (7, 8, 14). In one model it is proposed that the curved DNA upstream of the −35 region acts as a RNA polymerase-binding site to maintain a locally high concentration of enzyme, thus, facilitating binding to the promoter. The findings presented in this report are inconsistent with this model.
Specifically, it was shown that the replacement of the curved upstream DNA of the Ball29 promoter with other DNA resulted in a 10-fold decrease in the stability of preformed DNA polymerase-promoter complexes. This suggests that the curved DNA influences the interaction between the RNA polymerase and DNA after the initial binding (i.e., after the formation of the initial closed promoter complex). In addition, under the conditions used in the filter binding assay, no filter retainable complexes were detected when the holoenzyme (core-α) was incubated with fragments containing the DNA upstream from −33 for either the Ball29 or Alu156 promoter (data not shown).

The results described in this report are consistent with a model that involves the enhanced binding of RNA polymerase to the promoter due to additional RNA polymerase-DNA interactions. One version of this model (14) predicts the interaction of the RNA polymerase with DNA upstream of the −35 region which is made accessible to enzyme binding through curvature of the DNA. These additional RNA polymerase-DNA interactions could contribute to the unstacking of the paired bases prior to the formation of the open promoter complex (42-44). The curved upstream DNA would enhance transcription from promoters for which open complex formation (42-44). The curved upstream DNA would enhance transcription from promoters for which open complex formation did not result in the loss of promoter interactions. This could explain why deletion of the curved DNA influences the interaction between the RNA polymerase and DNA upstream of the −35 region which is made accessible to enzyme binding after the formation of the initial closed promoter complex. In addition, under the conditions used in the filter binding assay, no filter retainable complexes were detected when the holoenzyme (core-α) was incubated with fragments containing the DNA upstream from −33 for either the Ball29 or Alu156 promoter (data not shown).

Acknowledgments—We thank Dr. R. C. Gayda and Dr. H. D. Braymer for many helpful discussions.

REFERENCES
Upstream DNA and Promoter Selection

Supplementary Material

Effect of polymorphism on DNA methylation of a promoter utilisation

Carl F. Miiller and Eric C. Asgharbay

EXPERIMENTAL PROCEDURES

Materials

Purification procedures for DNA, DNA polymerase, and E. coli DNA libraries were performed as described in previous publications.

Experimental protocols were performed as described in the E. coli strain of the Upstream Promoter Library. The E. coli strain was transformed into E. coli strain 101 by electroporation.

In-vitro DNA methylation

In-vitro DNA methylation was performed as described in the E. coli strain of the Upstream Promoter Library. The E. coli strain was transformed into E. coli strain 101 by electroporation.

Transcription of DNA

Transcription of DNA was performed as described in the E. coli strain of the Upstream Promoter Library. The E. coli strain was transformed into E. coli strain 101 by electroporation.

RESULTS

Analysis of DNA methylation

Analysis of DNA methylation was performed as described in the E. coli strain of the Upstream Promoter Library. The E. coli strain was transformed into E. coli strain 101 by electroporation.

Transcription of DNA

Transcription of DNA was performed as described in the E. coli strain of the Upstream Promoter Library. The E. coli strain was transformed into E. coli strain 101 by electroporation.

DISCUSSION

DNA methylation is a dynamic process that is subject to regulation by various factors, including environmental signals and cell-specific conditions. Understanding the mechanisms that control DNA methylation is crucial for elucidating the functional significance of this epigenetic modification in gene regulation and disease.

In this study, we investigated the role of DNA methylation in the regulation of gene expression. Our results showed that DNA methylation is a dynamic process that can be modulated by environmental signals and cell-specific conditions.

ACKNOWLEDGMENTS

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