Specific Contacts between the Bacteriophage T3, T7, and SP6 RNA Polymerases and Their Promoters*

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The specificity and structural simplicity of the bacteriophage T3, T7, and SP6 RNA polymerases make these enzymes particularly well suited for studies of polymerase-promoter interactions. To understand the initial recognition process between the enzyme and its promoters, DNA fragments that carry phage promoters were chemically modified by three different methods: base methylation, phosphate ethylation, and base removal. The positions at which these modifications prevented or enhanced binding by the RNA polymerases were then determined. The results indicate that specific contacts within the major groove of the promoter between positions −5 and −12 are important for phage polymerase binding. Removal of individual bases from either strand of the initiation region (−5 to +3) resulted in enhanced binding of the polymerase, suggesting that disruption of the helix in this region may play a role in stabilization of the polymerase-promoter complexes.

The DNA-dependent RNA polymerases that are encoded by bacteriophages T7, T3, and SP6 are particularly well suited for studies of polymerase-promoter interactions. Each of the phage enzymes consists of a single species of protein of ∼100 kDa that is able to carry out all of the steps in the transcription process in the absence of any additional protein factors (for review, see Chamberlin and Ryan, 1983). The T7 RNA polymerase has recently been crystallized, indicating that detailed structural information will soon be available (Sousa et al., 1990).

Despite a high degree of structural similarity (as judged by the conservation of their amino acid sequences) the phage RNA polymerases are exquisitely specific for their own promoter sequences. Each of the phage promoters is related to a common 23-base pair consensus sequence that extends from −17 to +6 (see Fig. 1). A core nucleotide sequence that extends from −7 to +1 is highly conserved throughout the three promoter types, suggesting that this region interacts with shared features of the polymerases in a common manner. The promoter sequences differ significantly in the region from −8 to −12, suggesting that the base pairs in this region are important for specific promoter recognition. Studies with synthetic phage promoters have shown that the primary determinants of promoter specificity for the T3 and T7 enzymes involve the base pairs at positions −10 and −11; substitution of T3 residues at these two positions in the T7 promoter sequence prevents recognition by the T7 polymerase and enables transcription by the T3 RNA polymerase (Klement et al., 1990). A domain in the T3 and T7 RNA polymerases that is responsible for the recognition of these determinants has been localized, and preliminary crystallographic data suggest that this region of the polymerase is positioned within a putative DNA-binding cleft (Joho et al., 1990).

Considerable information is now available concerning polymerase-promoter contacts and the functional role of elements within the promoter. Studies with promoter mutants suggest that the promoter consists of a binding domain that extends from about −17 to −4 and an initiation domain that extends from about −4 to +5 (Chapman and Burgess, 1987; Schneider and Stormo, 1989; Klement et al., 1990). Base substitutions in the binding domain have a strong effect upon polymerase binding but relatively little effect on the rate of initiation. In contrast, base substitutions in the initiation domain have little effect upon polymerase binding but decrease the rate of initiation.

Footprinting of T3 and T7 RNA polymerase-promoter complexes with DNase I and methidium propyl-EDTA-Fe(II) has shown that the polymerase protects a region from about −21 to −3 upon binding (Basu and Maitra, 1986; Ikeda and Richardson, 1986; Gunderson et al., 1987). More recent experiments using the nonintercalating agent Fe(II)-EDTA provide greater resolution and have revealed two discrete protected regions located on one face of the DNA approximately one turn of the helix upstream from the start site (Muller et al., 1989). Although the protected regions flank the major groove, no contact with residues within the major groove in the area that lies between these regions has been demonstrated.

After binding to the promoter, the polymerase melts open a small region of the promoter around the start site, as judged by a hyperchromic shift (Oukley et al., 1979) and by the sensitivity of this region to attack by single-strand-specific endonucleases (Osterman and Coleman, 1981; Muller et al., 1989). In the presence of limiting amounts of substrate (ribonucleoside triphosphates) the methidium propyl-EDTA-Fe(II) footprint changes (Ikeda and Richardson, 1986; Gunderson et al., 1987). In the presence of GTP alone (which would permit the synthesis of a 3-nucleotide product) the

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polymerase protects the region from -21 to +8. In the presence of GTP and ATP (which would permit the synthesis of a 6-nucleotide RNA product) the region from -21 to +11 is protected. These results suggest either that there is a conformational change in the DNA-polymerase complex during the early stages of binding and initiation (Ikeda and Richardson, 1986) or that there is protection of the DNA template as a consequence of movement of the polymerase along the DNA during these stages (Muller et al., 1989).

Detailed structural information concerning specific contacts between the polymerase and promoter will become of considerable importance as crystallographic data begins to emerge. For this reason, a variety of approaches toward understanding polymerase binding will prove informative. In this work, we have refined the molecular model of the DNA surface that is contacted during the binding process through the use of methylation and ethylation interference techniques (Siebenlist and Gilbert, 1980). Our results demonstrate that specific residues in the major groove between positions -5 and -12 are important for polymerase binding. In addition, we also used missing contact analysis (Brunelle and Schleif, 1987) to identify bases whose removal affected promoter binding. Interestingly, elimination of bases from either strand of the initiation region (-5 to +3) resulted in enhanced binding of the RNA polymerase, suggesting that disruption of the helix in this region promotes local DNA unwinding and stabilizes the polymerase-promoter complexes.

The genes for the T3 and T7 RNA polymerases have previously been cloned and overexpressed, facilitating biochemical studies with these enzymes (Davanloo et al., 1984; Morris et al., 1986). In this report, we describe the cloning and expression of the SP6 RNA polymerase gene and a procedure for purification of the enzyme.

**MATERIALS AND METHODS**

**Preparation of T3 and T7 RNA Polymerases—**T7 and T3 RNA polymerases were purified from cultures of *Escherichia coli* BL21 carrying the plasmids pPAR1219 (Davanloo et al., 1984) or pCM56 (Morris et al., 1986), as previously described. The polymerase preparations had specific activities of \( \approx 3 \times 10^5 \) units/mg.

**Cloning, Expression, and Purification of the SP6 RNA Polymerase—**The region of the SP6 RNA polymerase gene that extends from a *HaeIII* site located 35 base pairs upstream of the start codon (the last 5 nucleotides of the recognition sequence for this enzyme are apparent in the sequence of Kotani et al., (1987)) to a *BstXI* site that
DNA polymerase. Cultures of BL21[pSR3] were propagated in Luria broth (Maniatis et al., 1982) containing 50 μg of ampicillin/ml to an absorbance of 0.8 at 600 nm. Isopropyl-1-thio-β-D-galactopyranoside was added to a final concentration of 0.4 mM, and after 4 h the cells were harvested by centrifugation. The cell pellet (3 g from 1 liter of culture) was washed once with 400 ml of HB (50 mM Tris-HCl, pH 8.2, 2 mM EDTA, 20 mM NaCl, 1 mM DTT), resuspended in 3 ml of HB, and stored at −70 °C. All subsequent steps were performed at 4 °C.

Frozen cells were thawed and the volume was adjusted to 100 ml of HB. Phenylmethylsulfonyl fluoride (0.1 mM), DTT (2 mM), and lysozyme (150 μg/ml) were added to the final concentration indicated, and the mixture was incubated at 0 °C for 20 min. The cells were lysed by the addition of sodium deoxycholate (0.05%), MgCl₂ (10 mM), and DNase I (50 μg/ml, Sigma), and the lysate was incubated for 20 min with occasional stirring.

Ammonium sulfate was added to a final concentration of 0.2 M by the addition of 5.25 ml of a 0.3 M solution, and the mixture was stirred for 10 min. The sample was centrifuged at 15,000 rpm for 20 min in a Sorvall SS34 rotor. One-tenth volume of 5% polyethyleneimine (BDH Chemicals, Ltd.) adjusted to pH 8 with HCl was added to the supernatant with constant stirring over a 1-min period, and stirring was continued for a further 15 min. The sample was clarified by centrifugation at 15,000 rpm for 30 min. A further 1.25 volume of buffer C containing 400 mM NaCl at a flow rate of 9.3 ml/h. Peak fractions (0.5 ml each) were pooled and dialyzed overnight against 2 volumes of storage buffer (20 mM KP04, pH 7.7, 1 mM EDTA, 200 mM NaCl, 50% (v/v) glycerol, 1 mM DTT). Polymerase assays (Morris et al., 1986) were carried out in a volume of 50 μl containing 2 μg of SP6 phase DNA. The final enzyme preparation had a specific activity of >10⁶ units/mg of protein (Butler and Chamberlin, 1982) and is >95% pure as judged by polyacrylamide gel electrophoresis (data not shown).

Preparation of Modified Promoter-containing DNA—Plasmid pJB30 contains the T3 φ10 promoter (map position, 54.5%; Bailey et al., 1983), pAR436 contains the T7 φ10 promoter (Studier and Rosenberg, 1981), and pSP65 (Promega Corp.) carries a class III SP6 promoter (Melton et al., 1984). All plasmids were propagated in E. coli HB101 (Maniatis et al., 1982). Plasmids were digested with restriction enzymes, and the resulting fragments were radioactively end-labeled using either [α-32P]dNTPs and the large fragment of E. coli DNA polymerase (PolIκ) or [α-32P]ATP and polynucleotide kinase (Maniatis et al., 1982). The DNA was subsequently treated with a second restriction enzyme to yield a mixture of fragments, one of which contained the promoter and was labeled at only one end.

Three types of modifications were used in our interference studies: base methylation, phosphate ethylation, and base removal. Methylation with dimethyl sulfate was performed as described by Siebenlist and Gilbert (1980). Methylation mixes containing 1 μg of DNA and

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**Fig. 3. Methylation interference experiments with the T3 φ10 promoter.** Experiments were carried out as described in the legend to Fig. 2. Panel A (template strand) shows results obtained with an EcoRI-HindIII fragment of pJB30 that contains the T3 φ10 promoter. Polymerase:promoter ratios were: lane 1, no polymerase; lane 2, 8:1; lane 3, 16:1; lane 4, 32:1. Panel B (nontemplate strand) shows results obtained with a HindIII-EcoRI fragment of pJB30. Polymerase:promoter ratios were: lane 1, no polymerase; lane 2, 16:1; lane 3, 32:1; lane 4, 64:1. Lane 1 (no polymerase) represents a G ladder for the strands indicated. Arrows indicate nucleotide positions where methylation results in interference with polymerase binding (−7 and −9 of the template strand). Note that, as in the case of the T7 promoter, methylation of the base at −5 of the template strand results in enhanced binding by the polymerase.

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**Fig. 4. Methylation interference experiments with an SP6 promoter.** Experiments were carried out as described in the legend to Fig. 2. Panel A (template strand) shows results obtained with a HindIII-SphI fragment of pSP65 that contains an SP6 promoter. Polymerase promoter ratios were: lane 1; no polymerase; lane 2, 25:1; lane 3, 50:1. Panel B (nontemplate strand) shows results obtained with a HindIII-SphI fragment of pSP65. Polymerase promoter ratios were: lane 1, no polymerase; lane 2, 25:1; lane 3, 50:1; lane 4, 100:1. Lane 1 (no polymerase) represents a G ladder for the strands indicated. Arrows indicate nucleotide positions where methylation results in interference with polymerase binding (−5 and −7 of the template strand; −9, −11, and −12 of the nontemplate strand).
Fig. 5. Summary of methylation interference data for the bacteriophage promoters. Panel A, SP6 promoter; panel B, T7 promoter; panel C, T3 promoter. Template strands are white; nontemplate strands are blue. Red atoms indicate N7 positions of guanine residues where methylation results in binding interference. Yellow indicates enhancement of binding as a result of methylation. All other guanine N7s are green. A white cross indicates the N7 of the base at the +1 position. Computer graphics were performed with the program Promodeler I (New England BioGraphics). Sequences shown are those presented in Figs. 2-4.

1 μl of dimethyl sulfate in 200 μl of 10 mM sodium cacodylate, pH 8.0, were incubated for 30 s at 25°C. The reactions were terminated and the DNA was purified as described. Unlabeled plasmid DNA containing no bacteriophage promoter sequences was used as a carrier for all precipitations in this study rather than tRNA, which can inhibit binding of the phage polymerases to DNA (Gunderson et al., 1987). Methylated DNA was stored frozen for up to 1 week at -20°C prior to use.

Ethylations were performed as described by Siebenlist and Gilbert (1980). Reaction mixes containing 1–5 μg of DNA in 200 μl of 10 mM sodium cacodylate, pH 8.0, and 200 μl of ethanol saturated with ethylisotrosooene were incubated for 30 min at 50°C. Following purification by repeated ethanol precipitation, the ethylated DNA was used immediately in interference experiments.

Base removal was carried out as described by Brunelle and Schleif (1987) except for the use of plasmid DNA as carrier as described above. Under the conditions used, each DNA molecule sustained, on average, less than one depurination event, as evidenced by the presence of the majority of the DNA in the unreacted position of the sequencing gel following piperidine cleavage.

Interference Experiments—Modified DNA was divided into 0.5-μg aliquots and incubated with different concentrations of RNA polymerase at room temperature for 20 min under conditions that allow binding but not initiation. Binding reactions contained 10 mM Tris-HCl, pH 7.5, 8 mM MgCl₂, 1 mM DTT, and 4% glycerol in a volume of 20 μl. Polymerase:promoter molar ratios ranged from 0:1 to 164:1, as indicated. Following incubation, the mixtures were loaded onto 15-cm, 5% polyacrylamide gels in a Tris borate EDTA buffer and electrophoresed at 200 V for 2–3 h (Maxam and Gilbert, 1980). Promoter-containing fragments that have modifications that interfere with promoter binding enter the gel and migrate anomalously whereas unbound fragments migrate normally. These promoter–polymerase complexes are relatively unstable in this gel system and dissociate during the electrophoresis run; for this reason, discrete bands that would correspond to the polymerase-promoter complex are not observed (Ling et al., 1989; Klement et al., 1990). Promoter-containing fragments that remained unbound at high concentrations of RNA polymerase were eluted from the gel, treated with piperidine to cleave the DNA at the site of methylation, and analyzed by electrophoresis.

The effects of methylation of the template strand of the T7 φ10 promoter are shown in Fig. 2B. Bands that become enriched as the polymerase concentration is increased indicate positions where methylation of G residues interferes with binding. Clearly, methylation of G residues at positions -7 or -9 on the template strand resulted in interference of binding. An unexpected finding was that methylation of the G residue at position -5 resulted in an unusually marked decrease in the intensity of the corresponding gel band with increasing polymerase concentration. This result indicates that methylation at this position enhances polymerase binding.

With regard to contacts with the nontemplate strand, the data of Fig. 2C show that methylation of the G at position -1 interferes with binding.

We see no effect on binding by methylation of the G residues at positions +1 to +3 and +5 on the nontemplate strand or at -12 on the template strand. As noted above, footprinting experiments have shown that the T7 RNA polymerase extends only to about -4 in the absence of substrate (Ikeda and Richardson, 1986; Gunderson et al., 1987). In light of this observation, it is not surprising that methylation of the G residues around the initiation site produced no inter-

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RESULTS

Methylation Interference—Plasmids that contain a phage promoter were digested with appropriate restriction enzymes, and the DNA fragments were end-labeled with ³²P. The DNA was then treated with dimethyl sulfate under conditions in which the N7 position of G residues is preferentially methylated. The modified DNA fragments were incubated with increasing concentrations of RNA polymerase and the enzyme/DNA mixtures were resolved by electrophoresis in 5% polyacrylamide gels (Fig. 2A). Under these conditions, restriction fragments that are bound by the polymerase migrate anomalously whereas unbound fragments migrate normally. As the polymerase:promoter ratio increases, the intensity of the band corresponding to the promoter-containing fragment decreases. The polymerase–promoter complexes are relatively unstable in this gel system and dissociate during the electrophoresis run; for this reason, discrete bands that correspond to the polymerase-promoter complex are not observed (Ling et al., 1989; Klement et al., 1990). Promoter-containing fragments that remained unbound at high concentrations of RNA polymerase were eluted from the gel, treated with piperidine to cleave the DNA at the site of methylation, and analyzed by electrophoresis.

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template strand indicates that the T7 RNA polymerase does base removal increases the efficiency of polymerase binding are pAR436. In both with a indicated (-5 to +4 on the template strand, -5 to +3 on the nontemplate strand) shows results obtained with an EcoRI-DdeI fragment of pJB3O. Polymerase:promoter ratios were:

| Lane | Polymerase:promoter ratio |
|------|---------------------------|
| 1    | no polymerase; lane 4, 8; 1 |
| 2    | lane 5, 16; 1 |
| 3    | no polymerase |
| 4    | the A and G marker reactions, respectively. |
| 5    | Lane 1 has been deliberately overexposed to enhance detection of weak bands. Arrows indicate nucleotide positions where ethylation of the sugar-phosphate backbone resulted in decreased binding of T3 polymerases (-5 to -7, -9, -14, and -15 of the template strand; -12 of the nontemplate strand).

**Fig. 6. Ethylation interference experiments with the T3 φ10 promoter.** DNA was modified by treatment with ethylnitrosourea and incubated with T3 RNA polymerase. Promoter-containing DNA fragments were resolved by electrophoresis as described in the legend to Fig. 2. Eluted fragments were cleaved by treatment with piperidine before electrophoresis in sequencing gels. Panel A (template strand) shows results obtained with an EcoRI-DdeI fragment of pAR436. Panel B (nontemplate strand) shows results obtained with an XbaI-SphI fragment of pAR436. In both panels, lanes 1 and 2 represent the G + A reactions, lanes 3 and 4, the T + C reactions. Polymerase:promoter ratios were:

| Lane | Polymerase:promoter ratio |
|------|---------------------------|
| 1    | no polymerase; lane 4, 8; 1 |
| 2    | lane 5, 16; 1 |
| 3    | no polymerase |
| 4    | the A and G marker reactions, respectively. |
| 5    | Lane 1 has been deliberately overexposed to enhance detection of weak bands. Arrows indicate nucleotide positions where ethylation of the sugar-phosphate backbone resulted in decreased binding of T3 polymerases (-5 to -7, -9, -14, and -15 of the template strand; -12 of the nontemplate strand).

The importance of contacts with the nontemplate strand upstream of -9 is also seen in experiments with the bacteriophage SP6 promoter (Figs. 4 and 5). In this case, methylation of G residues at -9, -11, and -12 in the nontemplate strand resulted in enhanced binding. No interference was observed when the G residues at -10 and -11 on the template strand were methylated. The latter result was surprising in light of previous experiments in which we demonstrated the importance of the bases at -10 and -11 to the specificity and efficiency of utilization of these promoters (Klement et al., 1990). However, as noted above, methylation at position -11 of the nontemplate strand of the T7 promoter resulted in significant inhibition of binding by this enzyme. This result suggests that the polymerases may make asymmetric contacts within the major groove (see Fig. 5). This interpretation is consistent with data from Martin and Coleman (1987) who constructed T7 heteroduplex promoters having a T3 base on either the template or nontemplate strand at -10. In these experiments, it was found that the T7 RNA polymerase would tolerate the T3 base at -10 on the template strand but not on the nontemplate strand.

**Fig. 7. Base removal experiments with the T7 φ10 promoter.** DNA was modified by treatment with formic acid (G + A reaction) or hydrazine (C + T reaction) and incubated with T3 RNA polymerase. Promoter-containing DNA fragments were resolved by electrophoresis as described in the legend to Fig. 2. Eluted fragments were cleaved by treatment with piperidine before electrophoresis in sequencing gels. Panel A (template strand) shows results obtained with a BamHI-DdeI fragment of pAR436. Panel B (nontemplate strand) shows results obtained with an XbaI-SphI fragment of pAR436. In both panels, lanes 1 and 2 represent the G + A reactions, lanes 3 and 4, the T + C reactions. Polymerase:promoter ratios were:

| Lane | Polymerase:promoter ratio |
|------|---------------------------|
| 1    | no polymerase; lane 4, 8; 1 |
| 2    | lane 5, 16; 1 |
| 3    | no polymerase |
| 4    | the A and G marker reactions, respectively. |
| 5    | Lane 1 has been deliberately overexposed to enhance detection of weak bands. Arrows indicate nucleotide positions where ethylation of the sugar-phosphate backbone resulted in decreased binding of T3 polymerases (-5 to -7, -9, -14, and -15 of the template strand; -12 of the nontemplate strand).

The usefulness of the methylation interference technique depends upon the presence of G residues at potential contact sites and is thus limited by the nature of acceptable promoter sequences. As more information can be obtained by looking at a variety of promoter types (see Fig. 1), we repeated the experiments above with the bacteriophage T3 and SP6 promoters and RNA polymerases.

Studies with the T3 RNA polymerase revealed an interference pattern similar to that observed in the T7 system (Fig. 3). Methylation at positions -7 and -9 on the template strand interfered with polymerase binding, and methylation at -5 resulted in enhanced binding. No interference was observed when the G residues at -10 and -11 on the template strand were methylated. The latter result was surprising in light of previous experiments in which we demonstrated the importance of the bases at -10 and -11 to the specificity and efficiency of utilization of these promoters (Klement et al., 1990). However, as noted above, methylation at position -11 of the nontemplate strand of the T7 promoter resulted in significant inhibition of binding by this enzyme. This result suggests that the polymerases may make asymmetric contacts within the major groove (see Fig. 5). This interpretation is consistent with data from Martin and Coleman (1987) who constructed T7 heteroduplex promoters having a T3 base on either the template or nontemplate strand at -10. In these experiments, it was found that the T7 RNA polymerase would tolerate the T3 base at -10 on the template strand but not on the nontemplate strand.
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tion of transcription is indicated by the polymerase binding was enhanced when bases in the region of removal of the bases in this region increases the binding specific endonuclease (Osterman and Coleman, 1981) is indicated by the RNA polymerase melts this region during 1987). The effects of these DNA modifications upon polymerase binding were explored using the T7 φ10 promoter. As can be seen from the data in Fig. 7, the removal of any individual base along the promoter was not sufficient to prevent polymerase binding. An unexpected result was that polymerase binding was enhanced when bases in the region from -5 to +6 is presented. • positions where methylation of G residues results in interference of binding; ○ positions where methylation leads to enhanced binding; □ ribose moieties that are protected from cleavage by Fe(II) (EDTA) (Muller et al., 1989); Δ, positions where ethylation of phosphates interferes with binding. The transcription start point and the direction of transcription is indicated by the open arrow. A region in which removal of individual bases leads to enhanced binding of the RNA polymerase is indicated in the major groove by X. The region of the nontemplate strand that is susceptible to cleavage by single-strand-specific endonuclease (Osterman and Coleman, 1981) is indicated by vertical arrows; a site of particular sensitivity is indicated by the large arrow.

DISCUSSION

In this report, we have probed the contacts of the phage RNA polymerases with their promoters by modifying the DNA with various chemical reagents and examining the effects of these modifications on polymerase binding. Three types of modifications were utilized: methylation, in which the N7 position of guanine is methylated through the action of dimethyl sulfate; ethylation, in which the phosphate groups in the sugar-phosphate backbone are ethylated by the action of ethynitrosourea; and base removal, in which individual bases are removed from the helical structure by limited treatment of the DNA with formic acid (depurination) or hydrazine (depyrimidinization). The results of these experiments and those of previous investigators are summarized in Fig. 8.

The data are consistent with the notion that the phage polymerases contact the promoter primarily on one face of the DNA helix and make numerous specific contacts in the region from -5 to -11. The results of the methylation interference studies suggest that multiple contacts with residues in the major groove are made in this region. If we assume that all three phage RNA polymerases contact their promoters in a similar fashion and that the DNA in this region is in the B conformation, our data suggest that the contacts in the major groove may be asymmetric, as the enzymes appear to be more susceptible to the presence of blocking groups on the non-template strand upstream from -9 and on the template strand downstream from position -9 (see Fig. 5).

The observation that the polymerases make close approaches to the base pairs at -11, -10, and -5 would appear to be incongruous with a "one-face" approach of the polymerase to the promoter because the N7 of the base at -5 would lie on the "back" face of the helix relative to the bases at -10 and -11. However, previous data suggest that the region from -6 to +2 is not in a B form in the polymerase-promoter complex, but is melted open (Osterman and Coleman, 1981; Muller et al., 1989). It may thus be possible for the polymerase to contact the N7 position of this base without wrapping around the helix.

In previous work, we also implicated the base pair at -15 in specific promoter recognition (Klement et al., 1990). The major groove contacts at this position would also be located on the back side of the DNA helix relative to base pairs at -10 and -11. Preliminary crystallographic data suggest that the putative DNA-binding cleft of the T7 RNA polymerase is large enough to accommodate the helix and would allow contacts on both sides of the promoter structure.

It is perhaps surprising that removal of bases in the binding region (-11 to -5) did not result in decreased polymerase affinity. One interpretation of this result is that the phage polymerases make multiple contacts in the binding region and that disruption of a single (or a few) contacts does not prevent binding. This is in contrast to interference experiments in which the insertion of a steric blocking group (by methylation or ethylation) disrupts multiple contacts. We cannot rule out the possibility that the loss of one contact in this region weakens the binding affinity of the polymerase for the DNA but that under our assay conditions the loss is not sufficient to prevent retardation of the restriction fragment in the gel binding assay.

The conditions used for methylation in our experiments (very short exposure to dimethyl sulfate) result predominantly in methylation at the N7 position of G residues, which lies in the major groove. Less extensive methylation of A residues at the N3 position in the minor groove may also occur under these conditions (Maxam and Gilbert, 1980), and in some of our experiments, we have observed limited cleavage at A residues (see, for example, Fig. 4B). Nevertheless, in none of the experiments that we performed did we see evidence for interference of binding as a result of methylation at A residues. This may reflect the low incidence of A methylation in our samples. Alternatively, our failure to observe interference may indicate that the polymerase is not making specific contacts with the A residues. As recently observed by Yang and Nash (1989). DNA-binding proteins seem to fall into two categories. The binding of most DNA-binding proteins is preferentially inhibited by methylation of G residues, presumably reflecting the importance of contacts in the major groove. The binding of proteins in the second group (such as integration host factor) is preferentially inhib-

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ited by methylation at A residues, reflecting the importance of minor groove contacts. Our results suggest that the phage polymerases fall into the former category. These results are seemingly in contrast to earlier work by Stahl and Chamberlin (1978) in which the nontemplate strand of a T7 promoter was repaired by the action of DNA polymerase in the presence of various base analogs that would introduce perturbations into either the major or minor grooves. These experiments demonstrated that some modifications, such as replacement of all adenine residues with 2,6-diaminopurine or all guanine residues with hypoxanthine (both of which affect the minor groove) prevented utilization of the modified promoter. We cannot exclude the possibility that specific minor groove contacts that we did not probe are important for polymerase binding. An alternative explanation for the observations by Stahl and Chamberlin is that contacts with minor groove elements are important during the initiation step, perhaps within the region that is melted open during initiation. As we have not measured the effects of base modifications on initiation in our experiments, we cannot exclude this possibility.

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