DNA strand specificity in promoter recognition by RNA polymerase⁺

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ABSTRACT

DNA strand and enzyme subunit specificities involved in the interaction between *E. coli* RNA polymerase and T7 DNA were studied by photo-crosslinking techniques. In non-specific enzyme-DNA complexes, subunits σ , β , and β' were crosslinked to both strands of the DNA. Under conditions leading to specific enzyme-promoter complexes, however, only σ and β subunits were crosslinked. The σ subunit was crosslinked preferentially to the non-sense strand at promoter sites. No such strand specificity was observed for the β subunit. These results provide insight into the molecular mechanism of promoter recognition and indicate that the interaction between RNA polymerase and DNA template is different at promoters and at non-specific sites.

INTRODUCTION

DNA-dependent RNA polymerase is the key enzyme responsible for the readout of all genetic information stored in the cell. Since only a small fraction of genes in the chromosome are expressed at any one time, it is crucial that in the first step of gene expression RNA polymerase recognizes proper sites on the DNA template where specific initiation of RNA chains can take place. These sites are called promoter sites. Although a great amount of information has been amassed on the molecular biology of gene expression, we are still far from understanding the molecular mechanism by which RNA polymerase recognizes promoter sites.

We have been investigating this problem using a simple system: the recognition of promoter sites on phage T7 DNA by *E. coli* RNA polymerase. The simplicity of this system is reflected in the fact that the selective transcription of T7 DNA by *E. coli* RNA polymerase is controlled at the level of enzyme-template interaction with no involvement of any other regulatory proteins (1). In order to understand the molecular mechanism of promoter recognition, it is essential to know the detailed structure of the enzyme-promoter complex. X-ray crystallography represents an ideal approach to

this problem, but this is technically impossible at the present time due to lack of suitable crystals and structural complexities of both *E. coli* RNA polymerase and T7 DNA. Another potential approach is high resolution electron microscopy. Although this technique has been used successfully to show RNA polymerase molecules specifically bound to promoter sites on DNA (2,3), the resolution has not been high enough to visualize any internal structures.

The approach that we adopted for studying the interaction of *E. coli* RNA polymerase with T7 DNA is photochemical crosslinking technique. This method "freezes" the contact points in the protein-nucleic acid complex by UV radiation thus permitting identification of the interacting regions of these molecules. In this manner, we have identified the subunits of *E. coli* RNA polymerase which are in close spatial proximity with T7 DNA (4). In non-specific enzyme-DNA complexes which occur in all regions of the DNA, subunits σ , β , and β' were crosslinked to DNA. In contrast, in the enzyme-promoter complexes only σ and β were crosslinked to DNA, while crosslinking of β' could not be demonstrated. These results clearly indicate that the interaction between RNA polymerase and DNA in nonspecific and promoter complexes are different in terms of the subunit specificity.

Another important question concerns the DNA specificity, i.e., which strand of T7 DNA does each subunit of *E. coli* RNA polymerase interact with during promoter recognition? The present communication addresses this question by further photochemical crosslinking studies. A preliminary account of this work has been reported elsewhere (5). Based on our results, possible models for the molecular topography of RNA polymerase-DNA complexes are proposed and their functional implications are discussed.

MATERIALS AND METHODS

Preparation of Enzymes

RNA polymerase was purified from *E. coli* B cells (grain processing corp.) by the method of Burgess and Jendrisak (6). Holoenzyme was further purified from a mixture of holo and core polymerase according to the procedure of Gonzalez *et al.* (7). The enzyme was 95-98% pure as judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. DNA polymerase I and exonuclease III were purified from *E. coli* B cells by the methods of Jovin *et al.* (8) and Richardson *et al.* (9) respectively. Both enzymes were electrophoretically pure and contained no endonuclease activity as assayed by the conversion of superhelical Col E1 plasmid DNA to the relaxed

(nicked) form. Restriction endonuclease MboI was purchased from New England Biolabs and was also a gift from Dr. J. Wetmur.

Preparation of DNA Template

³H-labeled and unlabeled T7 DNA were isolated from wild-type T7 phage grown in the thymine-requiring *E. coli* strain 011'B as described previously (4). Specific activity of [³H]T7 DNA was 200 cpm/pmol of nucleotide. T7 DNA with ³²P-labeled 3' strand and ³H-labeled 5' strand at both ends of the molecule was synthesized as follows. Homogeneously ³H-labeled T7 DNA was partially degraded with *E. coli* exonuclease III (1 unit per 10 µg DNA) to generate single-stranded 5' termini (9) in 0.01 M Tris-HCl (pH 8), 1 mM MgCl₂, and 10 mM 2-mercaptoethanol at 25° C. The extent of degradation was limited to 10% of total T7 DNA as determined from the acid soluble counts produced in the sample. The reaction was terminated by heating at 65° C for 5 min. The single-stranded 5' ends of the T7 DNA molecule were converted back to duplex DNA by *E. coli* DNA polymerase I (1 unit per µg exonuclease III-treated DNA) using [α -³²P] dATP (800 cpm/pmol) and the other three unlabeled deoxy-ribonucleoside triphosphates (75 µM each) in the presence of 10 mM MgCl₂. The repair synthesis was carried out at 20° C for about 1 hr and terminated by incubation at 65° C for 5 min. The modified DNA was purified by phenol extraction and was used within 2 days after synthesis.

MboI restriction cleavage of T7 DNA was carried out at 37° C for 1 hr using 1 unit of the restriction enzyme per 2 µg DNA as described by McDonnell et al. (10).

Filter Binding

Complexes between MboI-cleaved T7 DNA and RNA polymerase were formed in 0.02 M Tris-HCl (pH 8), 0.1 M KCl, 0.01 M MgCl₂, and 0.1 mM EDTA. The molar ratio of RNA polymerase to T7 DNA was varied from 0:1 to 20:1. Filtration through a millipore filter was carried out at room temperature immediately following a 5 min incubation of the enzyme and DNA at 37° C. The filtrate was electrophoresed on 1% agarose gel and stained with ethidium bromide to determine which fragments were retained on the millipore filter.

Photocrosslinking of the Enzyme-DNA Complexes

Photocrosslinking of the RNA polymerase-T7 DNA complexes was performed essentially as described previously (4). Enzyme-DNA complexes were formed as described for filter binding, and the samples were exposed to UV radiation at room temperature in 3 ml quartz cuvettes with 1-cm path length using a Mineralight UVSL-25 lamp operating at 254 nm. The exposure time was 20 min at 25° C with a surface energy density of 0.8 J/cm² for samples

containing strand-specific labeled DNA or 5 min with a surface energy density of 0.25 J/cm^2 for samples containing unlabeled DNA (see Figure 1).

Identification of the Cross-linked RNA Polymerase Subunits

The subunits of RNA polymerase photocrosslinked to DNA were identified as described previously (4). After nuclease digestion of free DNA, the photocrosslinked reaction products were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in phosphate buffer (11) or in discontinuous buffer system (12). The gels were cut into 2-4 mm slices, dissolved in 30% H_2O_2 and counted in a liquid scintillation counter to determine the appropriate radioactivity profile (^{32}P , ^3H , or ^{125}I) and its correlation to the gel pattern of RNA polymerase subunits. The identification of β' or β subunit was achieved by the characteristic cleavage patterns of these two subunits when limited proteolysis of RNA polymerase was performed with either trypsin or chymotrypsin (13). The proteolysis was carried out as previously described (4) with the following modification. To identify the β subunit, the photocrosslinked enzyme-DNA complexes were dialyzed against 8 M urea in 0.01 M Tris-HCl (pH 8), 1 mM EDTA for 4 hr at 37°C . The urea was then removed by dialysis at 4°C against 1 liter of 10 mM Tris-HCl (pH 8), 10 mM MgCl_2 , 0.1 mM EDTA, 5 mM dithiothreitol, 10% glycerol, and 0.1 M NaCl for 4 hrs with 2 changes of buffer. The denaturation and partial renaturation steps were necessary to form the isolated β subunit and $\alpha_2\beta$ subunit complex which are cleaved specifically by chymotrypsin to give the 80,000 and 70,000 dalton fragments of β subunit. Identification of the β' subunit by trypsin cleavage of the subunit into the 120,000 and 50,000 dalton fragments was carried out with the native enzyme. All other subunits were cleaved into small polypeptides which were not seen on the gel under the experimental conditions used.

Identification of the Crosslinked DNA Strand

Photocrosslinked unlabeled T7 DNA-RNA polymerase complexes were subjected to DNA strand separation by the poly (U,G)-CsCl density gradient centrifugation method (14) in the presence of 0.15% sodium dodecyl sarkosinate. During the heating and rapid cooling steps of DNA strand separation, sarkosinate was added to the sample to reduce the aggregation of enzyme subunits. After strand separation, the sample was dialyzed against 0.05 M sodium phosphate buffer (pH 8.0). The subunits of RNA polymerase cross-linked to the separated DNA strands were iodinated with Na^{125}I (17 Ci/mg, New England Nuclear) by the lactoperoxidase method using New England Nuclear radioiodination system. The sample was then loaded onto a DEAE cellulose

column and washed with 100 ml of 50 mM Tris-HCl (pH 8) and 0.1 M NaCl. The DNA and the crosslinked protein-DNA complexes bound to the column were then eluted with 0.7 M NaCl in 50 mM Tris-HCl (pH 8). The fractions containing DNA were diluted into or dialyzed against 50 mM Tris-HCl (pH 8) to reduce the salt concentration prior to nuclease digestion (see below).

RESULTS

We have used two different methods (Figure 1) with photocrosslinking to examine if there is DNA strand specificity in the interaction of *E. coli* RNA polymerase with phage T7 DNA in both promoter and non-promoter (random) complexes. The first method makes use of selective strand-specific labeling of T7 DNA with ^3H and ^{32}P before photocrosslinking to RNA polymerase, while the second method involves the separation of the two DNA strands after they have been photocrosslinked to the enzyme.

The Strand-Specific Labeling Method

Initial efforts to obtain T7 DNA template labeled with different radioisotopes in two strands involved isolation of individual T7 DNA strands followed by renaturation of the separated strands. Although part of the re-natured DNA produced a MboI restriction pattern very similar to that of native T7 DNA (10), its physical properties were grossly altered. We therefore selected a different approach of labeling the DNA strands by enzymatic modifications (Figure 1A). In this approach, the T7 DNA uniformly labeled with ^3H was partially digested with *E. coli* exonuclease III which degrades single strands starting at both 3' ends of a duplex DNA molecule. The modified DNA was then repaired by *E. coli* DNA polymerase I in the presence of [α - ^{32}P] dATP and three other deoxyribonucleoside triphosphates. In this way, the left end of the repaired T7 DNA molecule, which contains the major promoters of *E. coli* RNA polymerase (15, 16, 17), was doubly labeled with ^{32}P in the "sense" strand and ^3H in the "non-sense" strand. "Sense" strand is used here to denote the strand of T7 DNA which is utilized as template in specific *in vitro* transcription, while "non-sense" strand is its complimentary strand. Although the exonuclease and DNA polymerase used in these experiments were electrophoretically pure and contained no detectable endonuclease activity, the possible presence of nicks in the T7 DNA molecule could allow incorporation of ^{32}P into the non-sense strand in the promoter region due to "nick translation" catalyzed by DNA polymerase I (18). In order to rule out this possibility, the repaired T7 DNA was cleaved with restriction endonuclease MboI (10), and fragment B (0 to 20% of T7) and fragment C (90-100% of T7)

Figure 1. Protocols of the two methods used to identify specific interactions between DNA strands and RNA polymerase subunits. (A) Method involving differential labeling of T7 DNA with ^{32}P (wavy) in the sense strand and with ^3H (—) in the non-sense strand in the promoter region (P) before crosslinking to RNA polymerase. (B) Method involving strand separation of unlabeled T7 DNA and iodination of RNA polymerase after crosslinking of enzyme to DNA.

were isolated by agarose gel electrophoresis. These fragments were subjected to strand separation by poly (U,G)-hybridization and CsCl density gradient centrifugation (14) or polyacrylamide gel electrophoresis (19) after alkali denaturation of the DNA. Gel separation of the complementary strands was effective for fragment C but not for fragment B, while the poly (U,G)- CsCl

density gradient separation was more effective for fragment B. Both methods showed that ^{32}P was incorporated exclusively into the sense strand in fragment B and into the non-sense strand in fragment C (data not shown).

Specific *E. coli* RNA polymerase-T7 DNA complexes which formed at promoter sites were obtained by maintaining a low molar ratio (3:1) of enzyme to DNA. It has been shown that under these conditions, *E. coli* RNA polymerase binds preferentially to the three major promoter sites (A_1 , A_2 , A_3) located near the left-hand end of T7 DNA to form highly stable promoter complexes (20, 21, 22, 23). However, addition of RNA polymerase in excess of the amount needed to saturate the A promoter sites leads to stable binding of enzyme at three or four additional promoter sites (minor promoter sites B, C, D, and E) which are located at widely separated regions of T7 DNA. We have tested the binding of RNA polymerase to various MboI restriction fragments of T7 DNA using the nitrocellulose filter binding technique. As shown in Figure 2, at an enzyme-DNA molar ratio of 5:1 or lower, the B fragment is exclusively bound to RNA polymerase. However, at a molar ratio of 10:1 or higher, all the DNA fragments were bound to the enzyme. These results indicate selective binding of RNA polymerase to the promoter sites near the left-hand end of T7 DNA and that the enzyme does not form a tight complex with the minor promoter E on fragment C at a lower ratio of enzyme to DNA. Our findings are consistent with electron microscopic mapping of tight binding sites on T7 DNA for *E. coli* RNA polymerase (24, 25) which showed that at an enzyme to DNA molar ratio of 5:1, the RNA polymerase molecules were bound specifically to regions corresponding to the three major promoter sites. No significant binding was seen to occur at the region corresponding to the E promoter.

Non-specific enzyme-DNA complexes were formed by binding at a high molar ratio (12:1 or higher) of RNA polymerase to the MboI restriction fragment C of the *exo* III-treated, *pol* I-repaired T7 DNA. As mentioned above, the C fragment was labeled almost entirely with ^{32}P in the non-sense strand and ^3H in the sense strand in a fashion opposite to the labeling of the B fragment. The contribution from specific binding to the E promoter, if any, would be negligible due to a large excess of enzyme molecules bound non-specifically to the C fragment. Moreover, it has been shown by electron microscopy (3) that the binding of RNA polymerase to MboI fragment C under conditions similar to those used here is in a strictly random fashion and exhibits no specificity.

Specific and non-specific RNA polymerase-DNA complexes were exposed to

Figure 2. Binding of MboI restriction fragments of T7 DNA to *E. coli* RNA polymerase at various ratios of enzyme to DNA. A mixture of the T7 DNA fragments obtained by treatment of the DNA with MboI restriction endonuclease was incubated with RNA polymerase at various enzyme:DNA ratio as described in Materials and Methods. The sample was then passed through a nitrocellulose filter. The filtrate was electrophoresed in a 1% agarose gel and stained with ethidium bromide. The numbers above the gels represent the molar ratio of enzyme to DNA used in the experiment.

UV radiation at 254 nm to crosslink the enzyme to the DNA under identical conditions. After extensive nuclease treatment to degrade all of the DNA except the 30-40 base-pair DNA stretches protected by RNA polymerase, NaD₂SO₄-polyacrylamide gel electrophoresis was used to analyze the strand specificity of DNA contact with the individual subunits in RNA polymerase. As previously reported (4), the radioactivity from DNA co-migrated with the β , β' and σ subunits but not with the α subunit (Figure 3). While the β and/or the β' subunits crosslinked to both the ³²P- and ³H-labeled DNA strands in promoter as well as non-specific complexes, the σ subunit crosslinked exclusively to the ³H-labeled "non-sense" strand in promoter complexes (Figure 3A). Such strand-specific interactions of σ with DNA at promoter sites was not

Figure 3. Gel patterns and radioactivity profiles of NaDoSO₄-PAGE analysis of the photocrosslinked complexes of *E. coli* RNA polymerase with T7 DNA labeled with different isotope in each strand. Experimental details for preparation of the differentially labeled T7 DNA, formation of specific and non-specific enzyme-DNA complexes, photocrosslinking, and product analysis were described in Materials and Methods. The three gels are (A) specific complex formed by incubating 5 μ g of RNA polymerase with 50 μ g of differentially labeled T7 DNA for 5 min at 37 $^{\circ}$ C, (B) non-specific complex formed by incubating 5 μ g of RNA polymerase with 2 μ g of differentially labeled MboI fragment C of T7 DNA for 5 min at 37 $^{\circ}$ C, and (C) RNA polymerase without crosslinking. The Coomassie blue stained bands for subunits α , β , β' and σ are identified. The stained bands identified as N are nucleases used in the experiments to digest the unprotected DNA. In the radioactivity profiles, open bars represent 3 H counts and shaded bars represent 32 P counts. Dark bars represent 3 H counts after treatment of the crosslinked enzyme-DNA complexes with proteinase K.

observed in non-specific enzyme DNA complexes formed with MboI restriction fragment C (Figure 3B). That the radioactivity peaks in Figure 3 were actually due to small DNA pieces covalently crosslinked to RNA polymerase subunits were supported by the observation that degradation of the subunits with proteinase K prior to electrophoresis abolished these peaks (Figure 3A). In addition, when the UV-exposed, nuclease-treated enzyme-DNA complexes were purified by a glycerol gradient centrifugation in the presence of 0.5 M NaCl and then analyzed by NaDoSO₄-polyacrylamide gel electrophoresis, results identical to those in Figure 3 was observed.

Further evidence for the strand-specific interactions in RNA polymerase promoter complexes was obtained by comparing the photocrosslinking results from experiments using T7 DNA in which the major promoter region was uniformly labeled with ³H in both DNA strands (Figure 4A), in only the non-sense strand (Figure 4B), and in only the sense strand (Figure 4C). Comparison of Figures 4A and 4B shows that the absence of ³H label in the sense strand has little effect on the incorporation of radioactive counts into the σ subunit, while the incorporation of radioactive counts in the β and/or β' band decreases significantly. On the other hand, as shown in Figure 4C when the non-sense strand is unlabeled, the radioactivity peak associated with the σ subunit diminishes with a reduction in the β/β' associated counts comparable to that observed in Figure 4B. These results are in exact agreement with those expected for the specific interaction of σ subunit with the non-sense strand in the promoter complexes.

The Strand Separation Method

A second independent method (Figure 1B) was used to verify the photocrosslinking results obtained above. This method involves binding of RNA polymerase to native, unlabeled T7 DNA, and it uses a lower dose of UV exposure thereby reducing the possibility of artifacts due to modification or photo-damage of the DNA. After photocrosslinking, the DNA was heat-denatured in the presence of poly (U,G) and cooled rapidly. The two strands of T7 DNA, due to differential hybridization to poly (U,G), were separated by CsCl density gradient centrifugation (14). To identify the subunits of RNA polymerase crosslinked to each separated DNA strands, the protein was labeled with ¹²⁵I. The labeled photocrosslinked protein-DNA complexes were purified by DEAE cellulose chromatography and then subjected to nuclease treatment and electrophoretic analysis similar to those described in the first method.

Figure 5 shows the ¹²⁵I radioactivity profile of the photocrosslinked enzyme-DNA complexes obtained by the strand separation methods. In agreement

Figure 4. Radioactivity profiles of NaDoSO₄-PAGE analysis of the photo-crosslinked complexes of *E. coli* RNA polymerase with T7 DNA in which the major promoter region is uniformly labeled with ³H in both DNA strands (A), in the non-sense strand only (B), or in the sense strand only (C). Homogeneously ³H-labeled T7 DNA was obtained as described in Materials and Methods. T7 DNA in which the major promoter region was labeled with ³H only in the non-sense strand was prepared by partial digestion of homogeneously labeled T7 DNA with exonuclease III followed by repair with unlabeled dNTPs using DNA polymerase I. Similar methods were used for the preparation of T7 DNA in which the major promoter region was labeled with ³H only in the sense strand except that unlabeled T7 DNA was used as starting material and [³H]dATP was present during the repair of exonuclease III-treated DNA molecules. Other experimental conditions were the same as in Figure 3.

with the result from the differential labeling method, the σ subunit photo-crosslinks preferentially to the non-sense strand in the promoter complexes (Figure 5A) but to both the sense and non-sense strands in nonspecific complexes without detectable preference (Figure 5D). Also, the β and/or β' region has associated with it a radioactivity peak corresponding to both strands of the DNA, suggesting that either one or both of these subunits are in close contact to both DNA strands in specific as well as nonspecific complexes (Figure 5A and D). To further characterize the strand-specificity in the interaction of the β and/or β' subunit with the DNA template, we

Figure 5. Radioactivity profiles (^{125}I) of NaDoSO_4 -PAGE analysis of the photocrosslinked enzyme-DNA complexes using the strand separation method. Promoter complexes were formed by additions of 10 μg holoenzyme to 100 μg T7 DNA followed by a 5-min incubation at 37°C . Non-specific complexes were formed by additions of 10 μg holoenzyme to 5 μg T7 DNA (a molar enzyme:DNA ratio of 100:1) followed by a 5-min incubation at 37°C . Electrophoresis was performed in a discontinuous buffer (12). Other experimental details are as described in Materials and Methods except that 90 μg of T7 DNA was added to the non-specific complex after the UV irradiation as carrier for strand separation. Each panel shows the radioactivity profiles of ^{125}I -labeled RNA polymerase subunits photocrosslinked to the two separated strands of DNA which were electrophoresed in parallel on the same slab gel. Open bars represent the sense strand (with cpm values on the left ordinate) and shaded bars the non-sense strand (with cpm values on the right ordinate). The arrow indicates the direction of electrophoresis. The positions of subunit bands α , β , β' and σ are as indicated. The labels 120K, 50K, and 70K mark gel bands with molecular weights of 120,000, 50,000 and 70,000 daltons, respectively. (A) promoter complexes; (B) promoter complex treated with trypsin; (C) promoter complexes treated with chymotrypsin; (D) non-specific complexes; (E) non-specific complexes treated with trypsin; (F) non-specific complexes treated with chymotrypsin

have carried out limited proteolytic analysis of the photocrosslinked RNA polymerase-DNA complexes (see Materials and Methods). The results of the analysis with trypsin, which cleaves β' subunit into two fragments with molecular weights of 120,000 and 50,000 daltons, indicate that the β' subunit does not photocrosslink to either strand of the DNA in the promoter complex (Figure 5B) while it crosslinks indiscriminately to both DNA strands in the nonspecific complexes (Figure 5E). A similar analysis with chymotrypsin, which cleaves β into two fragments with molecular weights of 80,000 and 70,000 daltons, reveal that the β subunit crosslinks to both DNA strands in specific and nonspecific enzyme-DNA complexes (Figure 5C and F). The data presented in Figure 5 were derived from results obtained in one set of experiments. These results were reproducible qualitatively with only minor variations in peak heights. Furthermore, experiments in which 0.5 M NaCl was added to prevent the formation of enzyme-DNA complexes or experiments in which the enzyme-DNA complexes were formed but were not exposed to UV radiation gave only background radioactivity counts. These control experiments indicate that the radioactivity peaks associated with the enzyme subunits in Figure 5 were actually resulting from photocrosslinking of the subunits to the DNA in the enzyme-DNA complexes and not from subunits non-covalently attached to the DNA during the DNA strand separation due to heat induced aggregation or accidental comigration.

DISCUSSION

Although the exact mechanisms involved in UV photocrosslinking of proteins and nucleic acids are not well understood (25), several detailed studies have indicated the validity of this technique in probing specific molecular contacts in biological systems. Havron and Sperling (27) demonstrated that UV photocrosslinking of RNase A and its inhibitor uridine-2'(3')5'-diphosphate involves three amino acid residues of the protein which had been shown by x-ray crystallography to reside in the vicinity of the inhibitor binding site in the enzyme-inhibitor complex. Analogously, photocrosslinking of ATP to aminoacyl-tRNA synthetase was shown to involve a unique hexapeptide in the ATP binding site of the biologically active synthetase molecule (28). These established examples for the usefulness of UV photocrosslinking have prompted us to apply this method to studying the strand specificity of the *E. coli* RNA polymerase -T7 DNA interaction.

Nevertheless, there are certain limitations to the technique which must be considered when interpreting the data. First, a lack of photo-

crosslinking between two molecules does not necessarily mean that these molecules are not in close proximity because a crosslink would occur only when they are also photoreactive. However, it has been shown that photocrosslinking can take place between a large variety of amino acids with either purines or pyrimidines (27). Therefore, there is a strong likelihood of there being a photoreactive amino acid or nucleotide at or near the contact sites between nucleic acid and protein. Second, the efficiency of UV photocrosslinking with native DNA is low (a few percents). It is not possible to increase crosslinking efficiency by raising the radiation dosage because of the concomitant increase in UV damage to macromolecules. In spite of this difficulty, control experiments were carried out previously in our laboratory (4) which indicate that the photocrosslinking studies of T7 DNA to RNA polymerase similar to those described here represent the normal DNA-enzyme interaction.

The photocrosslinking results obtained by the two different methods described are in perfect agreement with one another. These results are summarized in Table 1. In the specific (promoter) complex, the σ subunit crosslinks exclusively to the non-sense strand while the β subunit crosslinks to

Table 1
Photocrosslinking of the E. coli RNA Polymerase-
T7 DNA Complexes

Complex	DNA Strand	Enzyme Subunits ^{1/}			
		α	β	β'	σ
Specific (promoter)	Sense	-	+	-	-
	Non-sense	-	+	-	+
Non-specific	Sense	-	+	+	+
	Non-sense	-	+	+	+

1/ A plus sign indicates that the subunit was cross-linked to the DNA strand, while a minus sign indicates that no crosslinking was detected.

both the sense and non-sense strands of the DNA. The β' and α subunits are not crosslinked to DNA at promoter sites. In contrast, only the α subunit does not crosslink to DNA in the non-specific complex; other three subunits (β , β' and σ) are all crosslinked to both DNA strands.

Our results clearly demonstrate that the interaction between RNA polymerase and DNA template is different at promoter and at non-specific sites not only in enzyme subunit specificity but also in DNA strand specificity. The most interesting finding concerns the σ subunit. It is the only subunit which appears to discriminate between the two DNA strands at the promoter sites, indicating that some of the information necessary for promoter recognition may reside in the σ subunit. Recent genetic studies with temperature-sensitive mutants (29, 30) have provided evidence for the involvement of σ subunit in transcriptional selectivity, perhaps by playing a critical role in promoter recognition. It has been suggested that σ may be involved in the opening of DNA strands by RNA polymerase (31, 32). Wang and Jacobson (33) have shown that binding of RNA polymerase holoenzyme but not core enzyme to λ DNA causes measurable unwinding of the DNA. A plausible model evolved from our photocrosslinking results is that at promoter sites the σ subunit interacts with only one strand of DNA. As a result of this selective interaction with the non-sense strand, the σ subunit may induce a local unwinding of the duplex DNA, thereby exposing the sense strand to serve as template to be transcribed by the catalytic subunit β . In other words, the function of σ subunit in the promoter recognition may be to act as a "local unwinding protein". This hypothesis is in accordance with the idea that the subunit plays a direct role in promoter recognition and utilization.

The interaction of the β subunit with the sense strand at promoter sites is expected since this subunit possess the substrate binding sites and is thought to contain the catalytic center of the enzyme (34, 35, 36). On the other hand, the interaction of the β subunit with the non-sense strand may at first seem surprising. However, it is likely that an anchoring (binding) site for the non-sense strand near the promoter may be required to prevent it from obstructing the transcription processes such as hydrogen bonding of the complementary nucleotide substrates to the sense strand. This requirement may be easily satisfied by placing the binding sites of both DNA strands on the β subunit.

Simpson (36) has examined the interaction between RNA polymerase and bromouracil substituted lac UV5 promoter by a combination of photocross-

linking and DNA sequencing techniques. Our results for the specific complexes are in agreement with those of Simpson's except for the β subunit. Our data indicate that the β subunit photocrosslinks to both the sense and non-sense strands, while Simpson's data show that β photocrosslinks only to the non-sense strand. This discrepancy could be due to the differences between lac UV5 and T7 promoters, or to alteration of native DNA conformation by bromouracil substitution (36). Furthermore, under the conditions used in Simpson's experiments, photocrosslinking can only occur at positions in which bromouracil has substituted for thymine (<50% incorporation). This could lead to a negative result in photocrosslinking between the β subunit of RNA polymerase and the sense strand of T7 DNA at the promoter site if the thymine residues near the contact points were not substituted by bromouracil.

For nonspecific complexes, all the enzyme subunits identified to be in close contact with the DNA (β , β' , and σ) were photocrosslinked to both strands of the DNA. One possible explanation for the lack of DNA strand specificity is that RNA polymerase binds to nonspecific sites on the DNA template in a nearly random fashion or in more than one orientation. Thus one may argue that at both specific and nonspecific sites, σ could interact with only one strand of the DNA template. The fact that it is crosslinked to both DNA strands in nonspecific complexes could be due to the possibility that it may bind in both orientation with respect to the longitudinal axis of DNA molecule. This possibility is unlikely when one considers the total radioactive counts (the sum of cpm for sense and non-sense strands) associated with the β/β' and σ subunits. Although both β and β' subunits crosslink to DNA in nonspecific complexes but only the β subunit crosslinks to DNA in specific complexes, the total counts associated with the β/β' band are roughly the same. Furthermore, the total counts associated with the σ bands are two-fold greater for nonspecific complexes than for specific complexes. These results would not be possible if the mode of interaction between RNA polymerase and T7 DNA were the same at both specific and non-specific sites. Also, it is conceivable that both β and β' subunits bind to the DNA at non-specific sites, or that RNA polymerase has two independent nonspecific DNA binding sites, one on β and the other on β' . It has been proposed (38) that a kinetically efficient mechanism for promoter search involves a series of direct DNA to DNA transfer events with the RNA polymerase molecule transiently bound between two DNA sites. The existence of two (or more) nonspecific DNA binding sites on RNA polymerase could provide a structural basis for such a mechanism.

The ability of RNA polymerase to form stable binary complexes at promoter sites on DNA has been well documented (1). The physico-chemical properties of these specific binary complexes are quite different from those of non-specific binary complexes formed on all regions of the DNA. The specific enzyme-DNA complexes are much more stable with half life of many hours (32), are more resistant to attack by heparin (39), and exhibit greater hypochromic shift as detected by UV difference spectroscopy (40, 41). In agreement with these observations, our photocrosslinking results indicate that the interaction of RNA polymerase with DNA template in specific and in nonspecific complexes is different. Furthermore, the present study provides some insight into the structural basis of these differences in terms of subunit and strand specificities for the RNA polymerase DNA template interaction. Such information has important implications for the molecular mechanism of gene transcription.

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Abbreviations used: Exo III, exonuclease III; J/cm², joules/cm²; Mbol, endodeoxyribonuclease purified from *Moraxella bovis*; NaDoSO₄-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; Pol I, *E. coli* DNA Polymerase I.

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