Interaction of T7 RNA Polymerase with DNA in an Elongation Complex Arrested at a Specific Psoralen Adduct Site*

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We have probed the interaction of T7 RNA polymerase with DNA in an elongation complex arrested by a site specifically placed psoralen diadduct or furan-side monoadduct using DNase I footprinting techniques. The psoralen derivative, HMT (4'-hydroxymethyl-4, 5', 8-trimethylpsoralen), was first placed at a specific site in the middle of a chemically synthesized double-stranded DNA fragment containing a T7 RNA polymerase promoter at one end. The psoralen molecule was photochemically attached either to 2 adjacent thymidine residues on opposite strands as a diadduct or to only 1 thymidine residue on the coding strand as a furan-side monoadduct. Using these psoralen-modified DNAs as templates for transcription, we found that T7 RNA polymerase was blocked at the psoralen adduct site and that the arrested elongation complex protected about 15 nucleotides upstream from the adduct on the coding strand and 20 nucleotides around the adduct on the noncoding strand from DNase I digestion. The two psoralen-modified DNA templates yielded identical RNA transcripts and DNase I footprints. In contrast, T7 polymerase protected only the coding strand from -20 to +8 in the initiation complex. These results suggest that the RNA polymerase undergoes a marked conformational change upon converting from an initiation complex to an elongation complex.

Bacteriophage T7 encoded RNA polymerase transcribes the class II and class III genes of the phage genome (Chamberlin et al., 1970; Chamberlin and Ryan, 1982). In contrast to Escherichia coli RNA polymerase, a multisubunit enzyme of \( M_r = 450,000 \) which binds to its promoter with an association constant of about \( 10^{11} \, \text{M}^{-1} \) (Roe and Record, 1985; Prosen and Cech, 1986), T7 RNA polymerase is a single polypeptide of \( M_r = 100,000 \) which binds weakly to its promoter with an association constant of \( \leq 10^4 \, \text{M}^{-1} \) (Chamberlin and Ring, 1973; Oakley et al., 1979; Ikeda and Richardson, 1986). The reduced stability of the T7 complex renders it sensitive to high ionic strength (Smeekens and Romano, 1986; Gunderson et al., 1987). DNase I and methidiumpropyl/EDTA/Fe footprinting experiments have shown that in the presence of the initiation nucleotide GTP, T7 RNA polymerase protects 28 nucleotides from -20 to +8 on the coding strand in the promoter-polymerase complex (Basu and Maitra, 1986; Ikeda and Richardson, 1986; Gunderson et al., 1987). Different results were obtained for the noncoding strand, as it was protected from methidiumpropyl/EDTA/Fe cleavage but not from DNase I cleavage. In the absence of GTP no specific DNase I footprint was observed (Basu and Maitra, 1986), although a weakly protected region from -21 to -3 on both strands was observed in the methidiumpropyl/EDTA/Fe footprinting experiments (Ikeda and Richardson, 1986; Gunderson et al., 1987).

A homogeneous population of elongation complexes is required in order to investigate the polymerase-DNA interaction during transcription. Using incomplete mixtures of ribonucleoside triphosphates, Ikeda and Richardson (1986, 1987) were able to stop transcription by T7 RNA polymerase after synthesis of short transcripts (\( \leq 15 \) bases in length) and obtained methidiumpropyl/EDTA/Fe footprints of the different transcription complexes as the polymerase (either the intact or a proteolytically nicked T7 RNA polymerase) moved downstream from the promoter. Here we report the DNase I footprints of homogeneous T7 elongation complexes which contain a 36-base long transcript. Blockage of transcription was accomplished by placing a site-specific psoralen monoadduct or interstrand psoralen cross-link downstream from a T7 RNA polymerase promoter. The monoaduct proved to be a noncoding lesion whereas the cross-link functioned as an absolute barrier to strand separation of the template DNA during elongation.

Psoralens are planar three-ring heterocyclic compounds which can intercalate between base pairs in double-stranded nucleic acids. Upon UV irradiation (320–380 nm), intercalated psoralens can photoreact with adjacent pyrimidine bases, mostly with thymidines in DNA and uridines in RNA, to form first monoadducts, which are linked to only one strand of the helix, and then diadducts, which are linked to both strands of the helix (for reviews, see Song and Tapley, 1979; Parsons, 1980; Cimino et al., 1985). The structures of the adducts formed between HMT\(^*\) (4'-hydroxymethyl-4, 5', 8-trimethylpsoralen) and thymidine are shown in Fig. 1. In the first step of the photoreaction, either a furan-side or a pyrane-side monoadduct is formed depending upon whether the 4',5'-double bond or the 3,4-double bond of the psoralen reacts with the 5,6-double bond of a pyrimidine base. By absorbing a second photon, the furan-side monoadduct can be converted into a diadduct if there is another adjacent pyrimidine base available for photoreaction on the other strand (Teusman et al., 1985; Shi and Hearst, 1987a). In contrast, the pyrane-side monoadduct cannot be driven to a diadduct because it does not absorb photons in the 320–380 nm wavelength region. All these adducts are photoreversible upon exposure to light in their respective absorption regions (Cimino et al., 1986; Shi and Hearst, 1987b).

* This work was supported by National Institutes of Health Grant GM 11180. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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4' Hydroxymethyl-4,5',8-trimethylpsoralen; \( M_r \) monomeric RNA polymerase; bp, base pair; EtOH, ethanol; NTP, nucleotide triphosphate.
The unmodified (138-mer), furan-side monooadducted (Mₐ, 138-mer), and the crosslinked (138-mer) double-stranded 138-mers were prepared exactly as described (Shi et al., 1987).

**Transcription and RNA Transcript Sequencing**

Transcription was modeled after the protocol described by Ikeda and Richardson (1986). The template (0.5 nM) was incubated with T7 RNA polymerase (0.09 μM) in 25 μl of T7-trans buffer (50 mM Tris-HCl, 10 mM MgCl₂, pH 8.0) in the presence of 0.2 mM ATP, 0.2 mM GTP, 0.2 mM CTP, and 10 μM [α-³²P]UTP (100 Ci/mmol) at 37°C for 10 min. The reaction was stopped by adding 100 μl of stop buffer (3.75 mM EDTA, 125 mM LiCl, 25 mM KCl, 3 μg of carrier tRNA, and 0.05% sodium dodecyl sulfate) followed by ethanol precipitation (3-fold EtOH at -20°C for overnight). The precipitated RNA transcripts were then dissolved in denaturing formamide containing 10 mM Tris-HCl, 1 mM EDTA, and 10 mM NaCl, pH 7.4, and analyzed on an 8% polyacrylamide, 8 M urea gel.

RNA sequencing reactions were performed as above except for the concentration of the nucleotide being analyzed. When [α-³²P]UTP was used as the labeled nucleotide, it was maintained at 10 μM (100 Ci/tmol) for all samples. The concentration of the nucleotide being analyzed was 6.7 μM ATP, 17 μM GTP, 0.2 mM CTP, or 13.4 μM CTP. The corresponding ³'cGDP, cGMP, and ³'cGMP concentrations were converted to 0.24 mM ATP, 0.2 mM GMP, 0.24 mM UMP, or 0.2 mM CTP, respectively. When [α-³²P]CTP was used as the labeled nucleotide, it was maintained at 10 μM (100 Ci/tmol) for all samples. The concentration of the nucleotide was 0.2 mM for analyzing ATP, GTP, CTP, and 0.7 μM for analyzing UTP.

Other conditions were identical as when [α-³²P]UTP was used as the labeled nucleotide. Sequencing reaction samples were treated and analyzed the same as above.

**DNase I Footprinting of Initiation and Elongation Complexes**

Initiation Complex—The initiation complex between T7 RNA polymerase and its promoter was formed by incubating the end-labeled template (0.5 nM) with the polymerase (0.22-0.75 μM) in 50 μl of T7-trans buffer at 37°C for 5 min in the presence of GTP, the first nucleotide of the transcript. The samples were then transferred to a room temperature water bath followed by the addition of 2 μl of calf thymus DNA (1 μg/μl) and 5 μl of 100 mM CaCl₂. DNase I digestion was initiated with the addition of 2.5 μl of 20,000-fold diluted DNase I (preincubated before use by diluting the stock enzyme in 30 mM NaOAc, pH 6.4, 25% glycerol). The reaction was terminated after 4 min by adding 2.5 μl of 0.25 M EDTA to the mixture and phenol extracting. (All phenol extractions in this paper consists of two equal volume phenol extractions, pH 7.8, one extraction of the phenol phase with H₂O to recover any DNA in that phase, and two equal volume ether extractions of the aqueous phases.) Finally, the solution was dried in a Speedvac concentrator and analyzed by electrophoresis on a 20% polyacrylamide, 7 M urea gel. The labeled DNA gel was autoradiographed directly on the wet gels. 5% gels were transferred to Whatman 3MM chromatography paper, dried in a Bio-Rad Model 483 Slab Dryer, and autoradiographed.

Elongation Complex—The end-labeled template (0.5 nM) was incubated with the polymerase (0.11–0.44 μM) in 50 μl of T7-trans buffer containing, unless otherwise indicated, 0.2 mM ATP, 0.2 mM GTP, 0.2 mM CTP, and 0.2 mM UTP at 37°C for 5 min. The samples were transferred to a room temperature water bath (22–24°C) followed by the addition of 2 μl of calf thymus DNA (1 μg/μl) and 5 μl of 100 mM CaCl₂. We have observed that the DNase I digestion is partially inhibited in the presence of high concentrations of NaCl. Consequently, in the presence of NaCl these digestions were carried out using 2.5–4 μl of 2,000-fold diluted DNase I, i.e. 12.5-fold more than that used in the absence of NaCl. The nucleic acid reaction was stopped after 4 min with 2.5 μl of 0.25 M EDTA and followed by phenol extraction. When Mₐ, 138-mer was the template, the samples were dried in a Speedvac concentrator and analyzed on 8% polyacrylamide, 8 M urea gel. When cross-linked 138-mer was the template, the samples were split into two equal portions. One was kept in the dark. The other was irradiated for 16 min in a 5.0 ml Eppendorf tube (covered with Reynolds Film 910 to cut off light below 240 nm) with 1 milliwatt/cm² 254 nm light from a low pressure germicidal lamp (estimated with a black-ray ultraviolet meter, Model J-225, short UV meter, Ultra-violet Products, Inc., San Gabriel, CA) to photoreverse the HMT diadduct. Both aliquots were taken to dryness in a Speedvac concentrator and analyzed by electrophoresis.
on an 8% polyacrylamide, 8 M urea gel. The gel was then dried and autoradiographed. DNase I footprinting of the elongation complex was also performed under the following conditions: transcription was performed at 37 °C followed by digestion at the same temperature for 4 min with 2 μl of 5000-fold diluted DNase I.

**Exonuclease III Digestion of Elongation Complexes**

Elongation complexes were formed under the same conditions as in the DNase I footprinting experiments. The complexes were then digested with 1 μl (65 units/μl) of exonuclease III at 37 °C for 10 min. The digestion was terminated by adding 2.5 μl of 0.25 M EDTA. The samples were then treated exactly as described for the DNase I-digested samples.

**RESULTS**

**Rationale and Template Construction**—A homogeneous population of elongation complexes at a specific site is required in order to study the interaction of the polymerase with DNA during elongation. It is known that during transcription RNA polymerase transiently unwinds the DNA and that the nascent RNA chain forms a short RNA-DNA hybrid with the coding strand (Von Hippel et al., 1984). A covalent interstrand cross-link blocks forward movement of RNA polymerase by preventing DNA unwinding and strand separation. When the cross-link is engineered to a site-specific location, the stalled transcription complexes are homogeneous and amenable to study by DNA footprinting techniques. Using such a complex it is also possible to determine the locations of the unwinding and the polymerizing sites of the enzyme relative to the DNA substrate.

As we described previously (Shi et al., 1987), a site-specific interstrand psoralen diadduct efficiently blocked transcription by E. coli RNA polymerase thereby providing a homogeneous population of arrested elongation complexes amenable to DNase I footprinting experiments. Employing the same strategy, we have prepared a double-stranded DNA with the coding strand (Shi et al., 1987), a site-specific interstrand psoralen diadduct efficiently blocked transcription by T7 RNA polymerase thereby providing a homogeneous population of arrested elongation complexes amenable to DNase I footprinting experiments. Employing the same strategy, we have prepared a double-stranded DNA with the coding strand.

**Fig. 2.** Sequence of the double-stranded 138-mer containing a T7 RNA polymerase class III promoter and the oligonucleotides from which the 138-mer was synthesized. The numbers above the sequence are relative to the T7 RNA polymerase transcription start site. The arrow indicates the thymidine residue on the bottom strand (coding strand) to which an HMT furan-side monoadduct is attached in the Mfu. 138-mer. The solid triangle indicates the thymidine residue on the top strand (noncoding strand) to which the pyrone-end of the HMT is attached in the cross-linked 138-mer. The furan-end of the HMT in the cross-linked 138-mer is attached to the thymidine residue indicated by the arrow on the bottom strand. Several restriction sites are also shown.

**Fig. 3.** Analysis of the transcripts from unmodified 138-mer, Mfu. 138-mer, and cross-linked 138-mer. The transcription and sequencing samples were electrophoresed on an 8% polyacrylamide, 8 M urea gel. 138-mer, 5'-end labeled template, which is not visible in lanes 4 (Mfu. 138-mer) and 5 (unmodified 138-mer) because much less template was loaded in these lanes and in lane 3 (cross-linked 138-mer) because the cross-linked 138-mer ran much slower and is not shown here. 110-mer; runoff transcript from unmodified 138-mer. Lanes 1 and 2; 3'-OCH3 RNA sequencing for UTP and CTP, respectively; [32P]UTP was used as the label. Lanes 3–5; transcripts from the cross-linked 138-mer, Mfu. 138-mer, and unmodified 138-mer, respectively. Lanes 6–9; 3'-OCH3 RNA sequencing for CTP, UTP, GDP, and ATP, respectively; [32P]UTP was used as the label. All samples had equal amounts of template but the amount of the transcripts loaded for the cross-linked 138-mer, Mfu. 138-mer, and unmodified 138-mer was, respectively, 1/6, 1/6, and 1/30 of the rest of the lanes.
transcription was completely blocked, whereas at low concentrations, transcript reactions yielded full-length runoff transcript (110 bases long). Since the DNA sequence was known, the RNA sequence could be easily determined from the UTP and CTP sequencing lanes.

Results in Fig. 3 indicate that transcription on the unmodified 138-mer yielded full-length runoff transcript (lane 5) while the furan-side monoadduct on the coding strand and the diadduct (lanes 4 and 3, respectively) blocked transcription by T7 RNA polymerase to yield identical aborted transcripts. The major transcript for both the M<sub>Pro</sub>,138-mer and the cross-linked 138-mer was the product of transcription termination at the HMT adducts, i.e. termination at UTP (+36). There were also two minor transcripts, corresponding to the addition of, respectively, one or two more bases after UTP (+36). Lowary et al. (1968) have observed that T7 RNA polymerase can insert a randomly selected residue to the 3'-end of the RNA chain before terminating, thus yielding a transcript longer than the expected runoff transcript. Our data suggests that this same phenomenon may occur in T7 elongation complexes blocked by a psoralen adduct. In the case of the unmodified 138-mer, transcripts containing one or two additional bases could not be resolved from the expected runoff transcript. However, we did observe transcripts running slower than the runoff transcript; the origin of these products is not clear.

**DNase I Footprints of the Initiation Complexes**—The interaction of T7 RNA polymerase with the synthetic promoter was probed by DNase I footprinting. The results obtained with the unmodified 138-mer (data not shown) demonstrate that the polymerase protects up to +8 on the coding strand (bottom strand). The protection disappeared when GTP, the first nucleotide of the transcript, was omitted or when 0.2 M NaCl was added. By contrast, there was no protection on the noncoding strand (top strand) in the presence of GTP or both GTP and ATP. These results are consistent with those reported for other T7 polymerase-promoter complexes (Basu and Maitra, 1986; Smeekens and Romano, 1986; Gunderson et al., 1987). The exact upstream boundary of the footprint on the bottom strand could not be determined due to the inability of DNase I to bind and incise near the termini of a helix. We assumed that the polymerase protected up to -20 at the upstream end based on previously reported results (Basu and Maitra, 1986; Ikeda and Richardson, 1986; Gunderson et al., 1987). Identical footprints were obtained for the binding of the polymerase to M<sub>Pro</sub>,138-mer (data not shown). This was expected since the HMT adducts in the M<sub>Pro</sub>,138-mer and cross-linked 138-mer lie outside the binding domain of the initiation complex. The above results are summarized in Fig. 8.

**DNase I Footprints of the Elongation Complexes**—Both the HMT furan-side monoadduct on the coding strand and the HMT diadduct efficiently blocked transcription by T7 RNA polymerase on the synthetic 138-mer (see above). Consequently, an arrested elongation complex could be formed using either HMT adduct. This complex, with a 5'-TP label on either the top strand or the bottom strand, was probed by DNase I footprinting. To maximize the yield of the elongation complex, transcription was conducted at low ionic strength (50 mM Tris-Cl and 10 mM MgCl<sub>2</sub>). DNase I footprinting was performed on the elongation complex after addition of NaCl to 0.2 M; this minimized the polymerase-promoter complex but had little effect on the elongation complex. If the M<sub>Pro</sub>,138-mer or unmodified 138-mer was used as the template, the digested samples were extracted with phenol, dried in a Speedvac concentrator, and analyzed on an 8% polyacrylamide, 8 M urea gel. Due to the interstrand nature of the HMT diadduct, fragments generated by DNase I cleavages 3' to the diadduct site in the cross-linked 138-mer elongation complex could not be identified because they were covalently attached to the other strand, which was also partially digested. Therefore, after the removal of protein by phenol extraction, half of each footprinting sample was irradiated with 254-nm light to reverse the cross-linked thereby allowing determination of the footprint 3' to the HMT diadduct. The samples (with and without irradiation at 254 nm) were analyzed on an 8% polyacrylamide, 8 M urea gel.

Fig. 4 shows the DNase I footprint of the bottom strand (coding strand) for the M<sub>Pro</sub>,138-mer elongation complex. Under the conditions described above, transcription was blocked at the HMT furan-side monoadduct, thus yielding an elongation complex with an RNA transcript approximately 36 bases long. The polymerase in the complex protected a region from +21 to +33 with enhanced cleavage at +19 and +20. The protection past +33, if any, could not be detected due to the inability of DNase I to cut in the vicinity of the complex. No protection was observed on the noncoding strand or the top strand. ATP, UTP, and CTP were omitted to prevent elongation. The protection was also sensitive to NTP concentrations as it can be seen that at 0.025 mM GTP and 0.01 mM ATP, UTP, and CTP the protection was very weak (Fig. 4, lane 5). Identical results were obtained when the bottom strand labeled cross-linked 138-mer was used as the template (Fig. 5).

When similar footprinting experiments were performed on top strand labeled M<sub>Pro</sub>,138-mer, the results shown in Fig. 6 were obtained. In contrast to the initiation complex, where no protection was observed on the noncoding strand, the polymerase in the elongation complex on the M<sub>Pro</sub>,138-mer template protected the noncoding strand for 20 bp (from +24 to +43) around the termination site. However, no enhancement was observed on the noncoding strand. As in the case of the coding strand, the protection was independent of the polymerase concentration from 0.11 to 0.44 μM but sensitive to the NTP concentration, as it can be seen that the protection was weaker at a NTP concentration of 0.05 mM (Fig. 6, lane 6). Identical results were obtained when both the complex formation and the DNase I digestion were performed at 37°C or when the cross-linked 138-mer was used as the template (data not shown). When the same experiments were performed using the unmodified 138-mer as the template, no protection was observed, consistent with the fact that transcription on this template yielded runoff transcript.

**Exonuclease III Digestion of the Elongation Complexes**—The 3' boundary of the protected region in an elongation complex can be delineated by digesting the complex with exonuclease III nuclease (a double-strand specific 3' to 5' exonuclease). Fig. 7 shows the results of such an experiment when the bottom strand labeled unmodified 138-mer or M<sub>Pro</sub>,138-mer was used as the template. In the case of the unmodified 138-mer, no elongation complex was detected (compare lanes 1 and 2). In contrast, when the M<sub>Pro</sub>,138-mer was used as the template, exonuclease III digestion of the DNA under the elongation conditions yielded a unique band corresponding to 3'-end digestion of the bottom strand to position +23 (Fig. 7, lane 4). This band did not appear when the T7 RNA polymerase was omitted (Fig. 7, lane 3). Instead, in this case, exonuclease III digestion yielded a product corresponding to the 3'-end digestion of the bottom strand to position +34, i.e. 3 bases from the HMT furan-side monoadduct. The +34 band produced in the presence of the polymerase was probably generated from uncomplexed M<sub>Pro</sub>,138-mer. These results indicate that the +23 band corresponds to 3'-end digestion of the bottom strand to the terminus of the elongation complex. Thus exonuclease III digestion yielded a 3' protection bound-
FIG. 4. DNase I footprints of the elongation complex on the bottom strand labeled MPY-138-mer. A, DNA sequencing (lanes G/A, G, C/T, C), in this figure as well as in other figures, were performed as described (Maxam and Gilbert, 1980). RNA polymerase was 0.22 μM. Lanes 1 and 6; control samples without RNA polymerase and DNase I digestion, respectively. GTP concentrations were 0.1, 0.1, 0.1, 0.05, 0.025, and 0.1 mM, respectively, for lanes 1-6. ATP, UTP, and CTP concentrations were equimolar at 0.1, 0.1, 0.02, 0.01, and 0.1 mM, respectively, for lanes 1-6. B, densitometric scans of lanes 1 (upper line) and 3 (lower line). The arrows indicate the boundaries of the protected region.

FIG. 5. DNase I footprints of the elongation complex on the bottom strand labeled cross-linked 138-mer. A, lanes 1'-6' are identical to lanes 1-6 except that they were irradiated at 254 nm to photoreverse the HMT diadduct. Lanes 1 and 4; control samples without RNA polymerase and DNase I digestion, respectively. Lanes 1, 3, and 4: 0.1 mM ATP, GTP, UTP, and CTP with 0, 0.22, and 0.22 μM RNA polymerase, respectively. Lane 2; identical to lane 3 except no ATP, UTP, or CTP added. Lanes 5 and 6; repeat of lanes 1 and 3, respectively. B, densitometric scans of lanes 5' (upper line) and 6' (lower line). The arrows indicate the boundaries of the protected region.

ary 2 bases downstream from that delineated by the DNase I protection experiments. An identical 3' protection boundary was determined when the cross-linked 138-mer was used as the template in the exonuclease III digestion experiment (data not shown).

When similar experiments were performed on the top strand labeled DNA templates, it was observed that exonuclease III nuclease frequently terminated in the right half of the DNA, which contained an E. coli RNA polymerase promoter (Shi et al., 1987), making the digestion patterns very difficult
**FIG. 6.** DNase I footprints of the elongation complex on the top strand labeled Mfe. 138-mer. A, lanes 1 and 3–5: 0.2 mM ATP, GTP, UTP, and CTP with 0, 0.11, 0.22, and 0.44 mM RNA polymerase, respectively. Lane 2; identical to lane 4 except no ATP, UTP, or CTP added. Lanes 6 and 7; identical to lane 4 except that nucleotide concentrations were 0.05 and 0.4 mM for lanes 6 and 7, respectively. B, densitometric scans of lanes 1 (upper line) and 3 (lower line). The arrows indicate the boundaries of the protected region.

**DISCUSSION**

Initiation Complex—DNase I footprinting on the initiation complex formed on the unmodified 138-mer template indicates that the polymerase protects up to +8 on the coding strand but affords no protection on the noncoding strand (Fig. 8), consistent with the results of Basu and Maitra (1986). The upstream boundary of the protected region on the coding strand was assumed to be −20 based on other reported results (Basu and Maitra, 1986; Ikeda and Richardson, 1986; Gunderson et al., 1987). The initiation complex formation requires the presence of GTP, the initiation nucleotide, and is inhibited by high NaCl concentration. These results and the transcription experiments on the synthetic template strongly indicate that the polymerase binds normally to the promoter in the double-stranded 138-mer. The data reported by Basu and Maitra (1986) and those in this paper show that the noncoding strand is not protected from DNase I cleavage in the initiation complex, in contrast to the methidiumpropyl/EDTA/Fe protection experiments reported by Ikeda and Richardson (1986) and Gunderson et al., (1987). The difference in the protection pattern is most likely due to the different probes used. Although the binding of the polymerase to its promoter does not protect the noncoding strand from DNase I cleavage, it can interfere with the intercalation of the methidiumpropyl group in the promoter region by either direct physical block-
age or unwinding and strand separation of the helix. Since intercalation is a prerequisite for DNA cleavage, its inhibition prevents nicking of both the coding and non-coding strands. DNase I footprinting experiments also show that the HMT addition in the M\textsubscript{Fuc} 138-mer and cross-linked 138-mer does not interfere with the polymerase-promoter interaction; this is as expected since the HMT adducts in these templates are outside the polymerase binding domain.

**Elongation Complex**—The site-specific HMT addition in the M\textsubscript{Fuc} 138-mer and cross-linked 138-mer efficiently blocked transcription by T7 RNA polymerase, thus generating an elongation complex containing a nascent RNA 36 or 37 bases long. The major RNA transcript was 36 bases long, which corresponds to transcription termination at the coding strand A (36) adjacent to the HMT-thymidine adduct. The minor transcript was one base longer. This RNA could have been generated either by transcription passing through the HMT moiety in the templates or by the nonspecific addition of a base to the 3' terminus of the RNA in the arrested elongation complexes. We believe that the former is unlikely since the HMT moiety is between the two A:T base pairs (36 and 37 bp) in both the M\textsubscript{Fuc} 138-mer and cross-linked 138-mer and the cross-linked template cannot be strand separated so as to allow the polymerase to synthesize RNA past the diadduct. Furthermore, it has been shown that T7 RNA polymerase can incorporate a randomly selected base at the 3' terminus of a runoff RNA transcript before the release of template and transcript (Lowary et al., 1986). Thus the elongation complexes formed on the M\textsubscript{Fuc} 138-mer and cross-linked 138-mer templates were heterogeneous in that the transcript in the complexes was either 36 or 37 bases long. The position of the polymerase on the template DNA in these two groups of complexes could either be shifted by one base from each other or occupy the same site relative to the HMT adduct. The latter is more likely if the 37-base long RNA is formed by the nonspecific addition of one base to the 3' terminus of the transcript in the arrested elongation complex.

The DNase I footprinting experiments indicate that the RNA polymerase in the elongation complexes on both the M\textsubscript{Fuc} 138-mer and cross-linked 138-mer templates protects 20 nucleotides (+24 to +43) in the vicinity of the HMT adduct on the noncoding strand and 13 nucleotides (+21 to +33) upstream from the HMT adduct on the coding strand (Fig. 8). In fact, the polymerase may protect the coding strand from +21 up to or even past the HMT adduct site (+37) since the RNA is synthesized up to that site in the complexes. Any protection around the adduct site, however, could not be detected due to the inability of DNase I to cut in the vicinity of the HMT furan-side monoadduct or diadduct (see Figs. 4–6).

The protection of the DNA template by the polymerase in the T7 elongation complexes was not as strong as that observed for the corresponding *E. coli* RNA polymerase complex (Carpousis and Gralla, 1985; Shi et al., 1987) and this protection weakened considerably when the NTP concentrations were ≤50 μM (see Figs. 4 and 6). The weak protection stems from the fact that T7 RNA polymerase elongation complexes are less stable than the analogous *E. coli* RNA polymerase complexes. It has been shown, for example, that *E. coli* elongation complexes can survive non-denaturating polyacrylamide gel electrophoresis and gel filtration (Rohrer and Zillig, 1977; Straney and Crothers, 1985; Shi et al., 1987). However, analysis of the T7 elongation complexes by non-denaturating polyacrylamide gel electrophoresis failed to detect any discrete nucleoprotein complex, although the DNA template was slightly retarded on the gel in the presence of the polymerase under both the initiation and elongation conditions (data not shown).

Based on the amount of RNA transcript synthesized from the M\textsubscript{Fuc} 138-mer and cross-linked 138-mer templates, we estimate that more than one copy of the transcript per template was synthesized during the 5-min incubation at 37°C under our standard transcription conditions, suggesting a lifetime for the arrested elongation complex of approximately 5 min or so. This short lifetime explains the weak protection of the template from DNase I digestion and the inability to detect any elongation complexes by nondenaturing gel electrophoresis.

Our results demonstrate that a dramatic conformational change occurs upon conversion of the initiation complex to the elongation complex. During initiation T7 RNA polymerase protects only the coding strand, whereas both strands are protected in the elongation complex. The size of the protected region also decreases upon conversion and is reminiscent of a similar contraction observed for the footprints of *E. coli* RNA polymerase complexes (Rohrer and Zillig, 1977; Carpousis and Gralla, 1985; Straney and Crothers, 1985; Shi et al., 1987). The structural significance of these changes remains to be elucidated.

Although the elongation complexes described here were generated using a site-specific HMT adduct to arrest the transcription process, the DNase I footprints of the complexes agree with those obtained by Ikeda and Richardson (1986), who used methidiumpropyl/EDTA/Fe to footprint a T7 RNA polymerase elongation complex stalled by omitting one ribonucleoside triphosphate. This agreement, which argues that the HMT adducts do not significantly perturb the structure of the arrested complex, is further supported by the following observations. First, a comparison of the DNase I cleavage patterns of the coding strand in the absence of T7 RNA polymerase indicates that the presence of the HMT adduct in the M\textsubscript{Fuc} 138-mer or the cross-linked 138-mer does not perturb the conformation of the DNA helix except in the immediate vicinity of the adduct (Shi et al., 1987; Figs. 4–6 and data not shown). Second, both the M\textsubscript{Fuc} 138-mer and cross-linked 138-mer elongation complexes yielded identical footprints even though the nature of the HMT adduct in each template is quite different.

The agreement between the protection of the DNA from DNase I digestion in our elongation complexes, which contain an RNA transcript 36 bases in length, and the protection of
the DNA from methidiumpropyl/EDTA/Fe cleavage in an elongation complex which contains an RNA transcript 15 bases in length (Ikeda and Richardson, 1986) suggests that transcription enters the elongation stage after synthesizing a transient 15 bases long. This is not apparent from Ikeda and Richardson’s data alone since they obtained a different protection pattern for each complex (containing nascent RNAs up to 15 bases long) examined.

The DNase I footprints of the elongation complexes formed on the M13-138-mer and cross-linked 138-mer templates have revealed two hypersensitive sites on the coding strand 17 and 18 nucleotides upstream from the HMT adduct site (Fig. 8). The HMT adduct and the DNase I hypersensitive sites may bracket a hypothetical unwound region encompassing approximately 17 bp, a conclusion in good agreement with similar experiments on the E. coli complex. Hypersensitive sites have been observed in the DNase I footprints of an E. coli RNA polymerase elongation complex arrested at a site-specific HMT diadduct site (Shi et al., 1987). It is known that during the elongation process, E. coli RNA polymerase unwinds approximately 12-17 base pairs of DNA template and maintains a short RNA-DNA hybrid helix between the nascent RNA chain and the DNA coding strand in the unwound region (Gamper and Hearst, 1982; von Hippel et al., 1984). Based on this, it has been proposed that the hypersensitive sites in the E. coli elongation complex correspond to the junction between the RNA-DNA helix and the reformed DNA-DNA helix (Shi et al., 1987). If similar DNA unwinding and RNA-DNA helix formation occurs during transcription by T7 RNA polymerase, the hypersensitive sites we have observed here would be at the postulated junction between the RNA-DNA helix and the reformed DNA-DNA helix.

Acknowledgments—We thank C. Noren and P. Schultz for their generous gift of T7 RNA polymerase and D. Sloane for help in densitometric scanning.

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