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Interactions of the RNA polymerase of bacteriophage T7 with its promoter during binding and initiation of transcription

("footprinting"/methidiumpropyl-EDTA/mRNA)

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ABSTRACT **Promoters for T7 RNA polymerase have a highly conserved sequence of 23 continuous base pairs located at position −17 to +6 relative to the initiation site for the RNA. The complex of T7 RNA polymerase with the phage ϕ10 promoter has been visualized indirectly by exploiting the ability of the polymerase to protect DNA sequences from cleavage by methidiumpropyl-EDTA·Fe(II). The DNA contacts made by T7 RNA polymerase have been mapped during binding and during the subsequent initiation of transcription. The RNA polymerase alone protects 19 bases in a region from −21 to −3. Synthesis of the trinucleotide r(GGG) expands the length of the sequence protected by the RNA polymerase and stabilizes the complex; 29 bases (−21 to +8) are protected, and the observed equilibrium association constant of the r(GGG) complex is 5×10^5 M^{-1}. The formation of a hexanucleotide mRNA, r(GGGAGA), further extends the protected region; 32 bases (−21 to +11) are protected. Finally, the synthesis of a pentadecanucleotide mRNA leads to a translocation of the region protected by the protein; the sequence now protected is reduced to 24 bases (−4 to +20).**

Gene *1* of bacteriophage T7 encodes an RNA polymerase that is responsible for the expression of the rightmost 80% of the T7 genome (1) and is also involved in the initiation of DNA replication (2–4). In contrast to the multisubunit RNA polymerases of bacteria and eukaryotes, T7 RNA polymerase consists of a single polypeptide of molecular weight 98,856 (5, 6). The physical and catalytic properties of the enzyme have been well documented (7, 8).

Seventeen T7 RNA polymerase promoters are present on the T7 DNA molecule (Fig. 1), and all are oriented for rightward transcription. The promoters consist of a highly conserved 23-base-pair (bp) sequence (9–11). The ϕ10 promoter, the promoter used in this study, is one of the strongest *in vivo* and *in vitro* (9); it has a sequence identical to that of the consensus sequence derived from all 17 promoters.

A useful tool for the identification of the DNA binding sites of small molecules and proteins is the protection of the nucleic acid from cleavage by enzymatic or chemical reagents. For example, the binding sites for netropsin (12), actinomycin (12), lac repressor (13), and *Escherichia coli* RNA polymerase (14–16) have been determined by using this "DNA footprinting" technique. Furthermore, in the case of *E. coli* RNA polymerase, sequential changes in the protected sequences reveal stepwise conformational changes of the enzyme during the initiation of transcription (15, 16).

The interaction of T7 RNA polymerase with its promoters is weak; the binding constant is estimated to be $<10^7$ M^{-1} (5, 17). Consequently, high concentrations of the enzyme are required to visualize the protection of specific sequences. In this communication we use overproduced T7 RNA polymerase (18, 19) to visualize the sequences protected from cleavage by methidiumpropyl-EDTA·Fe(II) [MPE·Fe(II)] (12, 20, 21) during binding of the promoter and initiation of transcription.

FIG. 1. Genetic map of bacteriophage T7: an illustration of the genome of T7 showing the RNA polymerase and its 17 promoters (ϕ). The sequence of promoter ϕ10 is identical to the consensus sequence derived from the 17 RNA polymerase promoters.

MATERIALS AND METHODS

DNA. Plasmid pRI10, a plasmid derived from pBR322 by the insertion of the T7 ϕ10 promoter, contains the *Cla* I/*Xba* I restriction fragment of T7 DNA from nucleotide 22,855 to 22,924 (18). To construct pRI10, flush ends were created on the *Cla* I/*Xba* I restriction fragment using *E. coli* DNA polymerase I; *Bam*HI linkers were ligated to the flush ends, and the fragment was inserted into the *Bam*HI site of pBR322. The ϕ10 promoter is oriented in a counterclockwise direction with regard to pBR322 (22). Plasmid pRI10 was amplified in the presence of chloramphenicol and isolated by CsCl equilibrium sedimentation in the presence of ethidium bromide (22).

Chemicals. Nucleoside triphosphates were obtained from P-L Biochemicals. $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ was obtained from Sigma, and methidiumpropyl-EDTA (12, 20, 21) was graciously provided by Peter B. Dervan (California Institute of Technology, Pasadena). [γ-^{32}P]rATP and [α-^{32}P]dCTP were obtained from ICN and New England Nuclear, respectively.

Enzymes. T7 RNA polymerase was isolated from *E. coli* HMS273 harboring plasmids pJL23 and pGP1-1 (18). The purified enzyme (1.18 mg/ml) has a specific activity (1) of 255,000 units/mg. The enzyme preparation was judged to be homogeneous by Coomassie blue staining of a NaDodSO$_4$/polyacrylamide gel. The protein content of the preparation was determined spectrophotometrically using a calculated extinction coefficient (23) of 130,430 $M^{-1} \cdot cm^{-1}$ at 280 nm. Restriction enzymes, large fragment of *E. coli* DNA polymerase I, and T4 polynucleotide kinase were purchased from New England Biolabs. Bacterial alkaline phosphatase was obtained from Bethesda Research Laboratories.

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Abbreviations: bp, base pair(s); MPE·Fe(II), methidiumpropyl-EDTA·Fe(II).

Isolation of a Fragment Containing the ϕ10 Promoter. The *Eco*RV/*Nar* I fragment of pRI10 containing the ϕ10 promoter was used in the protection studies. The ^{32}P label was introduced at the *Nar* I termini at either the 5′ end ([γ-^{32}P]ATP and T4 polynucleotide kinase) or the 3′ end ([α-^{32}P]dCTP and the large fragment of *E. coli* DNA polymerase I). After digesting the labeled DNA with *Eco*RV, the desired 312-bp restriction fragment was purified by gel electrophoresis. The fragment was eluted from the gel matrix with 0.6 M NH_4OAc, and the eluant was filtered through a 0.45-μm filter. The DNA was concentrated by ethanol precipitation in the presence of carrier tRNA (22). The fragment was then redissolved in water for use in the cleavage protection reactions.

Cleavage Protection Reactions. The reaction mixture contained 10 mM or 50 mM Tris·HCl (pH 8), 2 mM or 20 mM $MgCl_2$, 0.5 μg of tRNA, 20–50 ng of labeled DNA, 0–13 μg of T7 RNA polymerase, and, individually or in combination, rGTP, rATP, and rCTP at a concentration (each) of 400 μM. Specific conditions for each experiment are presented in the text and figure legends. After equilibration at 37°C for 10 min, 2–4 μl of a 0.1 or 0.05 mM solution of MPE·Fe(II) (12, 20, 21) were added. Three minutes later, 2.5 μl of 40 mM dithiothreitol were added, and the reaction mixture was incubated at 37°C for an additional 15 min. The reaction was stopped with 40 μl (1.3 vol) of 1% $NaDodSO_4$, 6 mM EDTA, and 200 μg of tRNA per ml. After the addition of 15 μl of a solution of 2.1 M NaOAc and 100 mM $MgCl_2$, the sample was extracted twice with a 1:1 (vol/vol) mixture of $CHCl_3$ and phenol, and the nucleic acid was precipitated with 5 vol of ethanol. The sample was redissolved in 90% formamide, and the DNA was electrophoresed through an 8% polyacrylamide denaturing gel (22). The gel was visualized by autoradiography; several different exposures were made to ensure that each autoradiogram was within the range of linear response for the XAR5 emulsion.

RESULTS

Visualization of Specific T7 RNA Polymerase/DNA Interactions. T7 RNA polymerase activity is highly sensitive to salt and magnesium concentrations; maximal activity occurs in 50 mM Tris·HCl (pH 8) and 20 mM $MgCl_2$ (5). Under these conditions, nitrocellulose retention experiments suggest that the interaction of T7 RNA polymerase with its promoters is weak, with a K_a of $<10^7$ M^{-1} (5, 17). One method that visualizes the site-specific binding of a molecule that is weakly associated with its recognition sequence is the protection of a specific DNA sequence from cleavage by a nonspecific nicking agent (12, 13). MPE·Fe(II) nicks double-stranded DNA nonspecifically (20); a molecule specifically bound to a DNA sequence inhibits the cleavage of the DNA by MPE·Fe(II), an event that is revealed by a gap in the autoradiograph of a sequencing gel (12, 21).

A 312-bp *Eco*RV/*Nar* I restriction fragment containing the T7 RNA polymerase promoter ϕ10 and uniquely labeled at one end was equilibrated with T7 RNA polymerase. MPE·Fe(II) was added, and nicking of the DNA was initiated with dithiothreitol. The resulting products were separated on an 8% polyacrylamide denaturing gel and visualized by autoradiography. The regions of the DNA fragment specifically protected by the bound T7 RNA polymerase appear on the autoradiographs as bands of reduced intensity (Fig. 2).

Binding Sites of T7 RNA Polymerase During Initiation of Transcription. We have used the protocol just described to study the binding of T7 RNA polymerase to the T7 ϕ10 promoter in reactions containing 50 mM Tris·HCl (pH 8), 20 mM $MgCl_2$, and either no nucleoside triphosphates, rGTP only, rGTP and rATP, or rGTP, rATP, and rCTP.

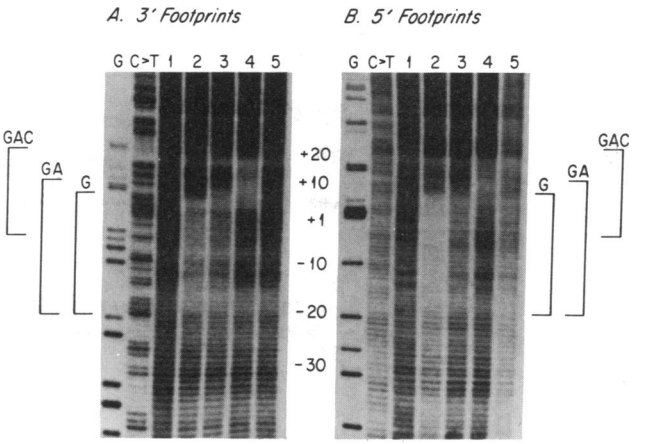

FIG. 2. Protection of promoter ϕ10 by T7 RNA polymerase during initiation of transcription. The patterns shown illustrate the protection of the 3′ labeled strand (antisense) (*A*) and the 5′ labeled strand (sense) (*B*) of promoter ϕ10. The G and C>T lanes contain the Maxam–Gilbert sequencing products of the specified reaction. The numbered lanes all contain 50 mM Tris·HCl (pH 8), 20 mM $MgCl_2$, 0.5 μg of tRNA, and 30 ng of the 3′ (*A*) or 5′ (*B*) labeled 312-bp *Eco*RV/*Nar* I restriction fragment. The individual lanes also contain 0 μg of RNA polymerase (lanes 1), 400 μM rGTP and 10.7 μg of RNA polymerase (lanes 2), 400 μM rGTP and rATP and 10.7 μg of RNA polymerase (lanes 3), 400 μM rGTP, rATP, and rCTP and 10.7 μg of RNA polymerase (lanes 4), and no nucleoside triphosphates and 10.7 μg of RNA polymerase (lanes 5). The brackets along the side of the gel that define the boundaries of the protected sequences for each observable binding site are determined by analysis of the densitometer tracings of the autoradiographs.

The first three nucleotides incorporated by T7 RNA polymerase at the ϕ10 promoter are all guanylates; consequently, in the presence of rGTP the trinucleotide r(GGG) is synthesized. In the presence of rGTP and rATP, the hexanucleotide r(GGGAGA) is synthesized, and in the presence of rGTP, rATP, and rCTP, the pentadecanucleotide r(GGGAGACCACAACGG) is synthesized.

When no nucleotide is present no specific protection of the ϕ10 promoter is observed. The addition of nucleoside triphosphates stabilizes the RNA polymerase·ϕ10 promoter complex. The stabilization of the RNA polymerase·promoter complex requires at least the initiation of RNA synthesis. None of the following nucleoside triphosphates will substitute for rGTP: 7-methylguanosine triphosphate, 7-methylguanosine(5′)ppp(5′)G, rATP, (α,β)methylene rATP, rCTP, or rUTP (data not shown). In addition, protection by T7 RNA polymerase does not appear with rGTP in the absence of Mg^{2+}.

In the presence of rGTP, the r(GGG)-initiated complex protects 27 bases on the 3′ labeled (antisense) strand and 29 bases on the 5′ labeled (sense) strand, defining a protected region from −21 to +8—±1 base at each border (Figs. 2 and 3). In the presence of rGTP and rATP (hexanucleotide synthesis), the sequence protected by the polymerase·promoter complex is unaffected at the 5′ end but extends 3 bases farther at the 3′ end. Thirty bases are now protected on the antisense strand, and 32 bases are protected on the sense strand, defining a protected region from −21 to +11 (Figs. 2 and 3). In the presence of rGTP, rATP, and rCTP (pentadecanucleotide synthesis), both ends of the protected region are translocated downstream. Twenty-three bases are protected on the antisense strand, and 24 bases are protected on the sense strand, defining a protected region from −4 to +20 (Figs. 2 and 3).

T7 RNA Polymerase Alone Binds Promoter Sequences Weakly. The lack of protection by T7 RNA polymerase in the absence of nucleoside triphosphates would appear to contra-

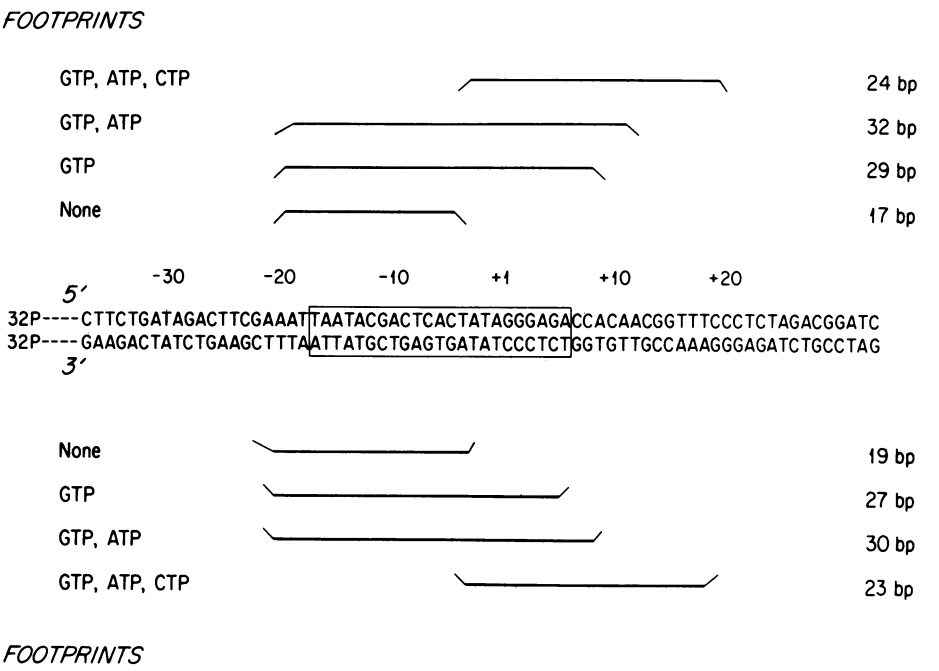

FIG. 3. Schematic illustration of the sequences protected by T7 RNA polymerase. The 23-bp conserved T7 promoter is boxed, and the numbering scheme begins +1 at the first nucleotide in the mRNA transcript. The brackets outline the regions protected by T7 RNA polymerase (±1 nucleotide at each boundary) in the presence of either no nucleoside triphosphates, rGTP only, rGTP and rATP, or rGTP, rATP, and rCTP. The height of each bracket is proportional to the efficiency of protection at that point in the sequence. Total sizes of the protected regions are given at the right.

dict previous studies (5, 17). Nitrocellulose retention of a polymerase·promoter complex in the absence of nucleoside triphosphates strongly suggested the existence of the specific complex (5, 17). We show here that the interaction of T7 RNA polymerase with the ϕ10 promoter in the absence of rGTP is too weak to protect the DNA from cleavage by methidium-propyl-EDTA under optimal conditions for transcription—50 mM Tris·HCl (pH 8) and 20 mM $MgCl_2$.

A simple strategy for increasing the general affinity of a DNA binding agent for its polyanionic target is to lower the overall concentration of the cations in the assay solution (24). While this study was in progress, S. Gunderson and R. Burgess (personal communication) informed us that protection of a promoter by T7 RNA polymerase in the absence of nucleoside triphosphates was possible at low salt concentrations. Using the protocol described above, we found that under altered conditions [50 mM Tris·HCl (pH 8), 2 mM $MgCl_2$, 0.5 μg of tRNA, 30 ng of the 312-bp restriction fragment, and 3.6 μg of T7 RNA polymerase] the T7 RNA polymerase protected 19 bases on the antisense strand and 17 bases on the sense strand, defining a protected region from −21 to −3 (Fig. 3). Interestingly, increasing concentrations of T7 RNA polymerase do not enhance the specific protection of the promoter. In fact, the addition of >3.6 μg of RNA polymerase causes the protected regions to gradually disappear on the autoradiograph (data not shown). Apparently, under reduced cation concentrations, the nonspecific, ionic binding of T7 RNA polymerase becomes appreciable, and this nonspecific binding effectively competes with recognition of the T7 promoter. This increase in nonspecific binding at low salt was also observed by Smeekens and Romano (28) in experiments comparing the nitrocellulose retention of specific and nonspecific complexes of T7 RNA polymerase with DNA.

Promoter Protection by the r(GGG)-Stabilized Complex Is Proportional to the Concentration of RNA Polymerase. In contrast to the similar affinity of T7 RNA polymerase for specific and nonspecific sites on DNA at low cation concentrations, the relative specificity for the promoter site is greatly increased at high concentrations of cations due to a reduction in the ionic binding to nonspecific sites. Consequently, under typical assay conditions, 50 mM Tris·HCl (pH 8) and 20 mM $MgCl_2$, the extent and definition of the protection of the binding site of the r(GGG)-stabilized polymerase·promoter complex increase monotonically with increasing amounts of T7 RNA polymerase (Fig. 4).

From this titration of the ϕ10 promoter with increasing amounts of T7 RNA polymerase, the ratio of the concentration of bound promoter to the concentration of free promoter can be determined from densitometer traces of the autoradiographs of the denaturing gels (Fig. 5). From these values the binding efficiency of T7 RNA polymerase to its promoter

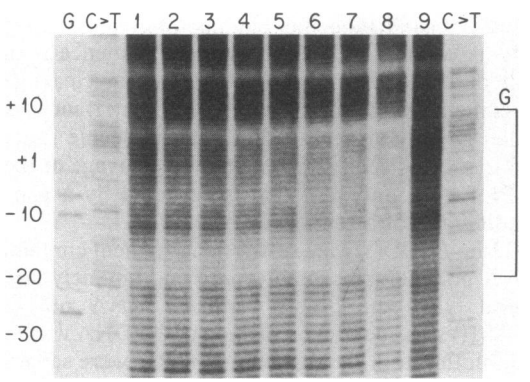

FIG. 4. Promoter protection as a function of increasing T7 RNA polymerase concentrations. Lanes 1–9 contain, in a 30-μl reaction mixture, 50 mM Tris·HCl (pH 8), 20 mM $MgCl_2$, 400 μM rGTP, 0.5 μg of tRNA, 20 ng of the 3′ ^{32}P-labeled 312-bp *Eco*RV/*Nar* I restriction fragment, and the following amounts of T7 RNA polymerase: lane 1, 3.6 μg; lane 2, 4.7 μg; lane 3, 5.9 μg; lane 4, 7.1 μg; lane 5, 8.3 μg; lane 6, 9.5 μg; lane 7, 10.7 μg; lane 8, 13.1 μg; and lane 9, 0 μg. The lanes marked G and C>T are Maxam–Gilbert sequencing samples of the labeled fragment. The bracket outlines the boundaries of the protected sequence.

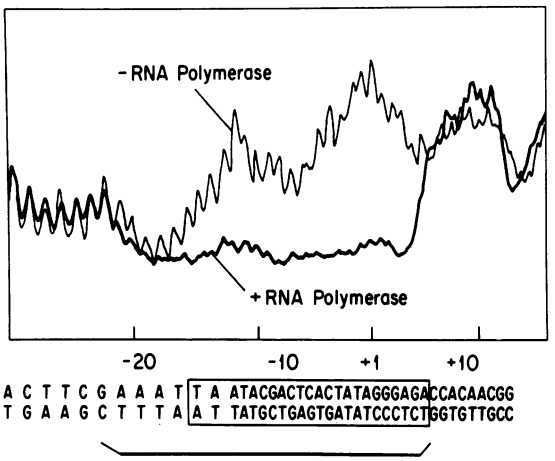

FIG. 5. Visualization of the promoter-specific binding site of the rGGG-stabilized complex of T7 RNA polymerase. Shown are reproductions of the Joyce–Loebl densitometer scans of lane 8 (lower trace) and lane 9 (upper trace) from the gel pictured in Fig. 4. The promoter is boxed and the numbering scheme begins at the initial nucleotide of the mRNA transcript. The protected region is also bracketed by a histogram as in Fig. 3.

can be directly related to the concentration of added polymerase at a constant concentration of rGTP. A plot of the ratio of bound promoter to free promoter versus the concentration of added polymerase yields a function with a slope of $5 \times 10^5\ M^{-1}$ (Fig. 6). The linearity of this plot suggests that the pathway to the initiated r(GGG) complex can be approximated with a "pseudo"-bimolecular mechanism (see *Discussion*).

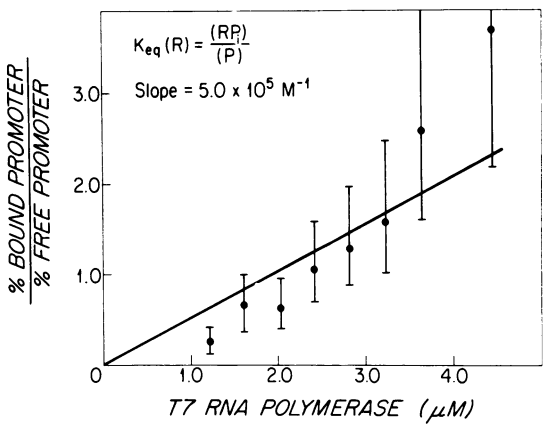

FIG. 6. Dependence of the ratio of the concentration of bound promoter to the concentration of free promoter on the concentration of T7 RNA polymerase. Quantitative determination of the extent of the protection of a given sequence was calculated after normalization of the intensity of the peaks of a given scan (see Fig. 5). The ratio of the integrated peak intensities of 10 bases from within the protected sequence to the sum of the integrated intensities of 6 bases 5′ to the protected region and 15 bases 3′ to the protected region was determined for each of the samples. For this ratio, the value corresponding to 0% protection ($ratio_0$) is determined by the sample/lane that contains no T7 RNA polymerase; a ratio value of 0 then corresponds to 100% protection. Any sample that returns a ratio value ($ratio_n$) between the two extreme values is then that fraction of the 0% value ($ratio_n/ratio_0$) unprotected and $1 - (ratio_n/ratio_0)$ protected. Furthermore, $(1 - ratio_n/ratio_0)/(ratio_n/ratio_0)$ is equivalent to the value given by the concentration of the promoter·polymerase complex divided by the concentration of free promoter, $[RP]/[P]$. Since $K_{eq} = [RP]/[R][P]$ for a simple bimolecular reaction, where K_{eq} is the equilibrium constant, $[RP]$ is the concentration of the promoter·RNA polymerase complex, $[P]$ is the concentration of free promoter, and $[R]$ is the concentration of free RNA polymerase, a linear relationship of $[RP]/[P]$ versus $[R]$ suggests bimolecular constraints and yields K_{eq} from the slope of the function. The slope of the plot is $5 \times 10^5\ M^{-1}$.

DISCUSSION

The general mechanism for the initiation of transcription can be illustrated with a sequential, multistep model (16, 25). Free promoter (P) and polymerase (R) associate to form a specific closed complex (RP_c). The trapping of RNA polymerase in a nonspecific complex is a nonproductive side reaction that is usually ignored. With T7 RNA polymerase this nonproductive interaction can also be ignored at moderate concentrations of cations (28). The closed complex then opens the strands of the promoter to form an initiation-competent open complex (RP_o). The open complex can now go on to initiate RNA synthesis (RP_i) and, finally, isomerize to a transcriptionally competent unit (RP_t).

$$R + P \rightleftarrows RP_c \rightleftarrows RP_o \rightleftarrows RP_i \rightarrow \rightarrow \rightarrow \rightarrow RP_t.$$

For T7 RNA polymerase the steps in the formation of the transcription unit can be visualized by observing the sequences protected from cleavage by MPE·Fe(II) during the initiation event. The initial closed complex protects 19 bases of the promoter from −21 to −3. With rGTP present the RNA polymerase stably opens the promoter and initiates RNA synthesis with the formation of a rG trimer. At this point the entire consensus sequence of a T7 RNA polymerase promoter is protected (29 bases from −21 to +8). The protected region has been extended 10 bases downstream without movement of the 5′ boundary at −21. With rGTP and rATP the formation of a hexanucleotide transcript within the initiation complex extends the protected region an additional 3 bases at the 3′ end of the promoter, again without altering the 5′ border; 32 bases are protected from −21 to +11. Finally, when a pentadecanucleotide transcript is synthesized in the presence of rGTP, rATP, and rCTP, the sequences protected by the transcription complex are translocated downstream to a region −4 to +20, and the number of protected nucleotides is reduced to 24.

From these observations it is apparent that T7 RNA polymerase undergoes a number of conformational changes as it progresses from the closed complex with the promoter to a transcriptionally active complex. The expansion and contraction of the number of bases protected by the RNA polymerase—from 19 bases for RP_c to a minimum of 22 bases for RP_o [the size of the RP_o complex is estimated from the data presented by Strothkamp *et al.* (26)], to 29 and 32 bases for two of the initiation complexes (RP_i), and back to 24 bases for a transcription complex (RP_t)—show that the transitions from one intermediate to the next involve a substantial topological adjustment of the enzyme on the DNA.

Although the number of different conformations of the T7 RNA polymerase·promoter complex illustrates an intricate multistep pathway for promoter-dependent initiation of transcription, portions of the pathway can be considered more simply. The linearity of the plot of [% bound promoter]/[% free promoter] versus added RNA polymerase suggests that at a constant 400 μM concentration of rGTP (≈2-fold higher than the K_m for rGTP), the formation of the polymerase and promoter complex that is stabilized by a r(GGG) could possibly be analyzed as a pseudo-bimolecular association where the promoter and polymerase have a defined equilibrium constant for association.

$$R + P \overset{K_{eq}}{\rightleftarrows} RP_i$$

$$K_{eq} = [RP_i]/([R][P]).$$

To estimate an equilibrium constant for the formation of the stabilized initiation complex from free polymerase and free promoter, three assumptions must be made. (*i*) Nonspecific interactions must be negligible, which, as noted previously, is only valid in the presence of moderate to high salt concentrations (28). (*ii*) Added RNA polymerase must approximately equal the amount free in solution. Since the least amount of polymerase added to a reaction is in 300-fold excess of the amount of promoter, the second assumption is also valid. (*iii*) The preparation of enzyme must be assumed to be 100% active.

The satisfaction of these considerations allows the plot (Fig. 6) of [% bound promoter]/[% free promoter] versus the concentration of added polymerase to be considered equivalent to the function $K_{eq}[R] = [RP_i]/[P]$, where K_{eq} is the equilibrium constant, $[R]$ is the concentration of free RNA polymerase, $[RP_i]$ is the concentration of the polymerase·promoter complex, and $[P]$ is the concentration of free promoter. The slope of Fig. 6 ($5 \times 10^5\ M^{-1}$) can be taken as an estimate of the binding strength of the trimer-stabilized initiation complex of T7 RNA polymerase. A binding affinity of $5 \times 10^5\ M^{-1}$ is weak, less than the corresponding value for an initiated *E. coli* RNA polymerase complex by a factor of $\approx 10^5$ (16, 25). However, this behavior is not unexpected; several studies had noted the relatively poor affinity of T7 RNA polymerase for a T7 promoter (5, 17).

In the absence of nucleoside triphosphates, an upper limit for the association constant for T7 RNA polymerase and the ϕ10 promoter in 50 mM Tris·HCl (pH 8) and 20 mM $MgCl_2$ can be estimated by using the same rationale as discussed above. Thirteen micrograms of T7 RNA polymerase in a 30-μl reaction mixture causes the intensity of the bands from -21 to -3 to decrease by an average of 10% (data not shown). This equates to an upper limit for the association constant of $3 \times 10^4\ M^{-1}$, reinforcing the notion of a weakly associated closed complex.

Comparison of T7 RNA polymerase to *E. coli* RNA polymerase reveals similarities and differences. Their similarities include specificity for a conserved promoter, a multistep mechanism for the initiation of transcription that is characterized by isomerization of the polymerase, and stabilization of the promoter complexes as polymerization of the RNA chain progresses (14–16, 25). The two polymerases are different not only in the stability of their respective closed complexes but also in the observations that T7 RNA polymerase protects 32 bases (maximum), whereas *E. coli* RNA polymerase protects $\approx$70 bases (14–16). The 3′ end of the region protected by T7 RNA polymerase moves before the 5′ end, whereas *E. coli* RNA polymerase initially moves the 5′ end of the region protected by the polymerase (16). Finally, the T7 closed complex does not protect the entire conserved sequence of the promoter, whereas the *E. coli* RNA polymerase protects the entire promoter (14–16).

This last disparity is surprising in that the closed T7 RNA polymerase·promoter complex protects only 60% of the conserved sequences of the promoter. The remainder of the promoter is not protected until the open and initiated complex is formed. It has been suggested previously that the 23-bp T7 promoter is overdetermined for the specification of a unique binding site for T7 RNA polymerase (27). Our results suggest that perhaps only the first 15 bases of the promoter are necessary for the specific binding of the polymerase. The protection of the remainder of the promoter during initiation might indicate that these downstream sequences are important for the separation of the strands of the DNA helix and the initiation of the RNA transcript. With such an arrangement of function, perhaps the T7 RNA polymerase itself is split into a series of functional domains.

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