Salt-Dependent Binding of *Escherichia coli* RNA Polymerase to DNA and Specific Transcription by the Core Enzyme and Holoenzyme†

Annemarie R. Wheeler,‡ A-Young M. Woody, and Robert W. Woody*

Department of Biochemistry, Colorado State University, Fort Collins, Colorado 80523

Received May 6, 1986; Revised Manuscript Received December 17, 1986

ABSTRACT: The interaction of the *Escherichia coli* RNA polymerase with several forms of DNA has been studied by difference absorption spectroscopy, protection against endonucleases, and limited, specific initiation. The core enzyme is able to open duplex poly[d(A-T)] in 10 mM KCl. The core enzyme binds to promoters in linear DNA in a salt-dependent manner, but it does not bind to the same promoters in supercoiled DNA. The binding of the core enzyme is not as tight as that of the holoenzyme. The holoenzyme initiates specific transcription from promoters in a salt-dependent manner. The core enzyme also initiates specific transcription from the same promoters at approximately one-fifth the level of the holoenzyme with a different salt dependence. The profile of the salt dependence of specific transcription initiation varies with the promoter. The origin of differences between holoenzyme–DNA and core enzyme–DNA interactions and the mechanism whereby σ improves transcriptional specificity are discussed in light of these data.

DNA-dependent RNA polymerase is the enzyme responsible for transcription. In *Escherichia coli*, the enzyme is composed of five subunits ($\alpha_2\beta\beta'\sigma$). While the holoenzyme has been widely studied, the individual roles of the core enzyme ($\alpha_2\beta\beta'$) and the σ subunit are poorly understood, due to several experimental difficulties (Reisbig et al., 1981; Shaner et al., 1982). The generally accepted model for the action of RNA polymerase (Chamberlin, 1974), based on studies with the holoenzyme, postulates a series of steps in which the polymerase recognizes and binds the promoter with the aid of σ, locally melts the DNA, and initiates transcription. σ is proposed to dissociate from the T4 DNA ternary complex after the first several nucleotides have been incorporated (Travers & Burgess, 1969). On poly[d(A-T)], after approximately 10 ribonucleotides have been polymerized (Hansen & McClure, 1980a,b), σ is released and can bind to a free core enzyme, and transcription can be reinitiated. Little information is available about the interaction of the core enzyme with DNA. This study presents new information about how the holoenzyme and core enzyme differ in their interactions with DNA and in their limited specific transcription.

The interaction of core enzyme and holoenzyme with DNA has been studied by difference absorption spectroscopy, protection of DNA against restriction endonuclease digestion, heparin challenge, and specific, limited initiation. Difference absorption spectroscopy was employed to measure the opening of double-stranded DNA by the core enzyme. The salt dependence of specific and nonspecific binding of core enzyme and holoenzyme to linear and supercoiled DNA were assessed by their protection of DNA against restriction endonuclease digestion. The relative strengths of promoter binding of core enzyme and holoenzyme were determined by heparin challenge to binding. The salt dependence of initiation at the same promoters by the holoenzyme and core enzyme was studied by specific, limited transcription.

Materials and Methods

Materials. Calf thymus DNA and poly(dA) were purchased from Sigma Chemical Co. Poly[d(A-T)] was purchased from Pharmacia P-L Biochemicals, Inc. pBR322 was isolated from *E. coli* by the method of Birnboim and Doly (1979). *E. coli* MRE600 cells were purchased from Grain Processing Co., Muscatine, IA. Bovine serum albumin was purchased from Bethesda Research Laboratories. All other chemicals were reagent grade, and the water was deionized and distilled.

Purification of RNA Polymerase. RNA polymerase was isolated from *E. coli* MRE600 by the procedure of Burgess and Jendrisak (1975) with the modifications of Reisbig et al. (1979). Core polymerase was obtained by the procedure of Lowe et al. (1979) with minor modifications. The NaCl concentration was reduced to 0.05 M for loading and washing the Bio-Rex 70 column, and the core enzyme was eluted with a gradient of 0.2–0.6 M NaCl.

RNA Polymerase Activity Assays. Enzyme activity was assayed according to the method of Reisbig et al. (1979). The activity of the holoenzyme and core enzyme was determined under conditions similar to those used for the spectroscopic studies. The transcriptional activity on poly[d(A-T)], calf thymus DNA, and *Hin*dIII-linearized pBR322 was determined in 5-min assays. The assay solutions contained 0.1 mM ethylenediaminetetraacetic acid (EDTA),[1] 0.4 mM each of the required nucleoside triphosphates (NTPs), 50 nM enzyme, 500 pM [^{3}H]UTP, 0.1 mM DNA, 1 mM dithiothreitol, and one of the buffer systems listed in Table I. The results are summarized in Table I.

† This research was supported in part by U.S. Public Health Service Grant GM-23697. From a thesis submitted by A.R.W. to the academic faculty of Colorado State University in partial fulfillment of the requirements for the degree of Ph.D.

* Author to whom correspondence should be addressed.

‡ Present address: Department of Biochemistry, University of Minnesota, St. Paul, MN 55108.

[1] Abbreviations: Δ229, 229-base-pair DNA fragment; Δ587, 587-base-pair DNA fragment containing the β-lactamase promoter; EDTA, ethylenediaminetetraacetic acid; HEPPS, *N*-(2-hydroxyethyl)-piperazine-*N*′-3-propanesulfonic acid; NTPs, nucleoside triphosphates; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane; units, nanomoles of NTPs incorporated per milligram of enzyme in 10 min.

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Table I: Transcriptional Activity of RNA Polymerase Holoenzyme and Core Enzyme[a]

		poly[d(A-T)]		calf thymus DNA		pBR322[b]	
buffer	enzyme	37 °C	25 °C	37 °C	25 °C	37 °C	25 °C
1[c]	holo	5600	3000	530	310	510	220
	core	5600	3500	280	180	40	20
2[d]	holo	5000	3800	470	170	470	300
	core	4200	2400	220	120	50	30
3[e]	holo	5000	3500	530	210	350	190
	core	4000	2300	320	120	50	20
4[f]	holo	5000	2300	460	220	260	110
	core	4200	1700	300	120	50	10

[a] Units are nanomoles of UTP incorporated per milligram of enzyme in 10 min. [b] *Hin*dIII-linearized pBR322. [c] 10 mM $MgCl_2$, 100 mM KCl, 10 mM Tris. [d] 10 mM $MgCl_2$, 100 mM KCl, 10 mM HEPPS. [e] 5 mM $MgCl_2$, 10 mM KCl, 10 mM HEPPS. [f] 5 mM $MgCl_2$, 10 mM HEPPS.

The concentration of active RNA polymerase was determined by using the method of Chamberlin et al. (1979) with a few modifications. The template used was a 229-base-pair fragment containing gene II promoter of M13 phage, and the synthesized RNA was analyzed as described by Reisbig et al. (1979). The fractions of enzyme capable of initiation were 75% and 50% for holoenzyme and core enzyme, respectively.

Spectroscopic Determinations. The concentrations of the DNAs were determined by absorption, with the extinction coefficients given by Reisbig et al. (1979), except for pBR322 (6.65 mM^{-1} cm^{-1} at 260 nm). RNA polymerase core enzyme and holoenzyme concentrations were determined by absorption, with the following extinction coefficients: core enzyme, 227 mM^{-1} cm^{-1}, and holoenzyme, 309 mM^{-1} cm^{-1}. These extinction coefficients were determined from the previously reported $E^{1\%}$ values of 5.8 and 6.7 (Levine et al., 1980), assuming molecular weights of 385 000 and 464 000 for the core enzyme and holoenzyme, respectively.

Buffers were passed through a 0.45-μm Millipore filter and degassed. Enzymes were passed through a Bio-Gel P-6DG column, equilibrated with the working buffer, just before their use. Absorption spectra were obtained on a Cary 118C scanning spectrophotometer. Difference absorption spectra were obtained by using matched, double-chamber, rectangular cells with a chamber path length of 0.437 cm. The temperature was controlled by a Lauda circulating water bath. Spectra were stored and analyzed by using a MINC 11 minicomputer (Digital Equipment Corp.). Spectra were smoothed by the least-squares method (Savitzky & Golay, 1964).

Endonuclease Protection. To detect the nonspecific binding of core RNA polymerase to linear double-stranded DNA, *Hin*dIII-linearized pBR322 (6 nmol) was incubated at 37 °C for 10 min with and without core polymerase (60 pmol) in 1 mM HEPPS, pH 7.9. The $MgCl_2$ concentration was 0.4–8 mM, and the chloride ion concentrations were adjusted to 5–70 mM with KCl. *Hae*III was added, and the 150-μL mixture was incubated at 37 °C for 2 h. After phenol/$CHCl_3$ and ether extractions and ethanol precipitation, the samples were fractionated by 10% PAGE and then stained with ethidium bromide.

The binding of polymerase to promoters in linear DNA was studied by the above method, using *Ava*I-linearized pBR322 under the conditions given in Figure 3. Negatives of the ethidium bromide stained gels were scanned densitometrically on a Beckman DU-8B spectrophotometer, and peak areas were computed by integration.

The binding of polymerase to supercoiled promoters was studied under the same conditions as for the linear DNA as indicated in Figure 4. The data were analyzed by using the same methods as for the linear DNA except that the peak areas were determined by cutting and weighing the peaks.

Plasmid Fragment Formation. The 229-base-pair fragment (Δ229) containing the gene II promoter from M13 phage was isolated from pM13/AI25 (gift of Paul Hagerman) by cleavage with *Eco*RI according to the directions of Bethesda Research Laboratories (BRL). The 587-base-pair fragment (Δ587) containing the β-lactamase P3 promoter (West & Rodriguez, 1980) was isolated from pBR322 by cleavage with *Hae*III according to the directions of BRL. The restricted DNAs were phenol/$CHCl_3$ and ether extracted and ethanol precipitated. Isolation of the desired fragments was based on the procedure of Johnson and Malcolm (1982), using BRL's preparative gel electrophoresis system.

Heparin Challenge. Twenty-microliter samples containing 83 μM Δ587, 270 nM core enzyme or holoenzyme, 40 mM Tris, pH 7.9, 10 mM $MgCl_2$, and either 75 or 180 mM KCl were incubated at 37 °C for 15 min. Heparin was added to the mixtures (0.1 g/μL), and they were incubated for 1 min before fractionation by 4% nondenaturing PAGE at 150 V for 5 h and stained with ethidium bromide.

Polymerase Binding Competency Assay. Twenty-microliter samples containing 370 μM poly[d(A-T)], 2 μM core enzyme or holoenzyme, 10 mM Tris, pH 7.9, and either 10 or 100 mM KCl were incubated at 22 °C for 15 min. The samples were fractionated by 4% nondenaturing PAGE at 100 V for 6 h and stained with Coomassie blue or ethidium bromide.

*Specific Transcription Assays. Pst*I-linearized pBR322 containing the P2 and P3 promoters or Δ229 were preincubated with core enzyme or holoenzyme (two polymerases per plasmid or fragment) at 37 °C for 10 min with dinucleotide initiator in buffers of the desired salt concentrations (see Figures 6–8) in an initial volume of 15 μL. Transcription was started by adding 5 μL of a nucleotide-containing cocktail of the same buffer and salt concentrations, containing cold and [α-^{32}P]NTPs (see Figures 6–8). Reactions were stopped after 5 min by adding 20 μL of stop buffer (10 M urea, 25 mM EDTA, 0.1% bromphenol blue, 0.1% xylene cyanol, 20% glycerol). For determination of the RNase A digestion products of the transcripts, 1 μL of 10 mg/mL RNase A was added after 5 min of transcription and allowed to digest the transcripts for 5 min before the stop buffer was added. The reaction mixtures were boiled for 2 min, transferred to ice for 5 min, and analyzed by urea-PAGE [25% acrylamide, 0.8 % bis(acrylamide), 7 M urea] run in Tris–borate buffer (45 mM Tris–borate, pH 8.0, 1 mM EDTA) for 4–6 h at 600–800 V. All assays using a given promoter were performed simultaneously, but the core enzyme and holoenzyme transcripts were loaded on separate gels. X-ray film was exposed to both the holoenzyme and core enzyme gels at the same time for the same length of time for comparison of the holo and core transcription. Additional autoradiographs were made to assess the salt dependence of the initiation. All autoradiographs were analyzed as described previously, and the areas were nor-

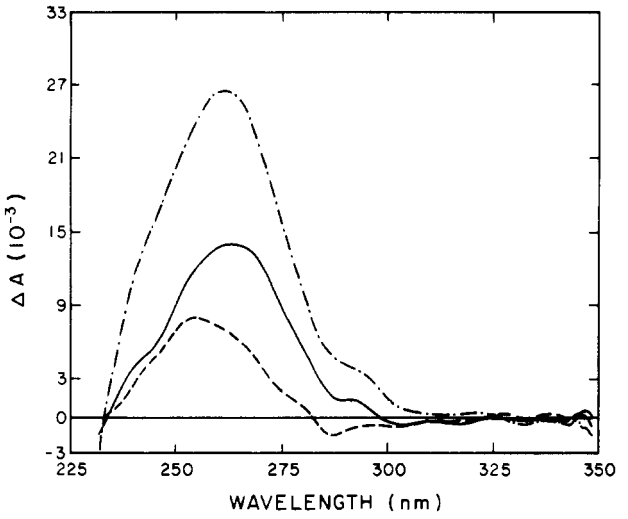

FIGURE 1: UV difference absorption spectra of poly[d(A-T)] titrated with core and holo RNA polymerases in 10 mM KCl and 1 mM HEPPS, pH 7.9, vs. DNA in 100 mM KCl and 1 mM HEPPS, pH 7.9 at 25 °C, with a poly[d(A-T)] concentration of 117 μM and enzyme concentration of 585 nM: (---) DNA alone; (—) core enzyme; (-·-) holoenzyme.

malized to an arbitrary constant for a given promoter.

RESULTS

Core Polymerase Opens Double-Stranded Poly[d(A-T)]. There has been no physicochemical study performed to address the question of whether the core enzyme is capable of opening double-stranded DNA to any appreciable extent. We have examined the interaction of core and holo RNA polymerases with double-stranded poly[d(A-T)] by ultraviolet difference absorption at low salt concentration (10 mM KCl), where aggregation and light scattering are minimized (Reisbig et al., 1981; Shaner et al., 1982), and the enzymes are transcriptionally active. Since the melting point of poly[d(A-T)] is lowered significantly at this low salt concentration, KCl was added to the reference DNA compartment to raise the concentration to 100 mM KCl, where the DNA is essentially 100% double stranded over the temperature range studied. The DNA was then titrated with holoenzyme and core enzyme over a concentration range of 150–400 base pairs per RNA polymerase from 9 to 35 °C. The partial melting of the DNA due to the low salt concentration caused a hyperchromicity and blue shift to 252 nm in the absorption spectrum of the DNA (Figure 1). Addition of core enzyme or holoenzyme caused additional hyperchromicity and a red shift to 262 nm. The fractional melting of the DNA was calculated from eq 1 of Reisbig et al. (1979), which assumes that the opened DNA on the surface of the enzyme has the same hyperchromicity as thermally denatured DNA. Totally melted poly[d(A-T)] was taken to have a 40% hyperchromicity compared to the double-stranded form (Allen et al., 1972). The fraction of DNA melted at the low salt concentration (Figure 2b) was subtracted from the fraction melted with polymerase present to give the minimum, fractional melting due to polymerase binding. The results are summarized in Figure 2a. At 10 °C, the extents of opening due to holoenzyme and core enzyme were approximately 3.5 and 2 base pairs per polymerase, respectively. At 35 °C the extent of opening increased to 7 and 5 base pairs per polymease for holoenzyme and core enzyme, respectively. Regardless of the actual number of base pairs opened, which depends on the comparison with thermal melting, the extent of opening is comparable for holoenzyme and core enzyme. These values represent the lower limit of base-pair opening since all enzyme molecules were assumed

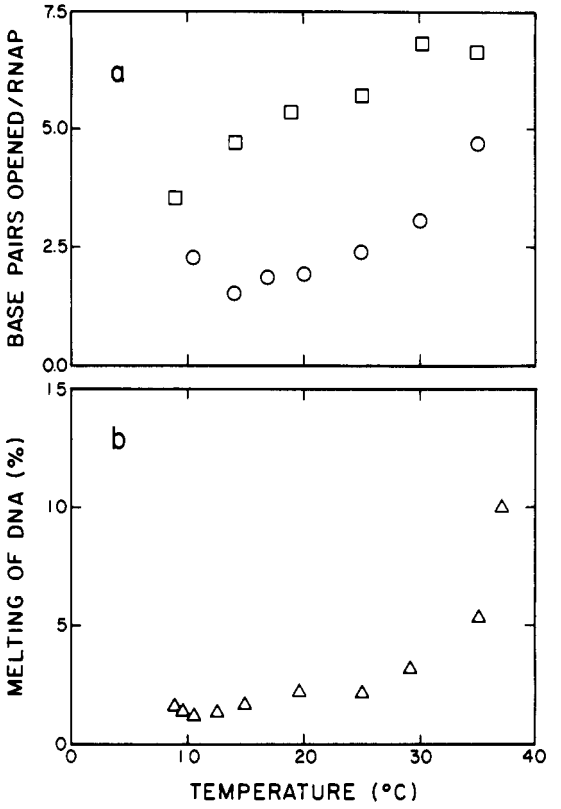

FIGURE 2: Melting of poly[d(A-T)] in the absence and presence of RNA polymerase vs. temperature: 117 μM poly[d(A-T)] in 10 mM KCl and 1 mM HEPPS, pH 7.9, vs. 117 μM poly[d(A-T)] in 100 mM KCl and 1 mM HEPPS, pH 7.9. (a) Base pairs opened per RNA polymerase beyond thermal melting of DNA. 146–390 nM RNA polymerase. (□) holoenzyme; (○) core enzyme. (b) Percentage melting of poly[d(A-T)] in 10 mM KCl vs. 100 mM KCl.

to be capable of binding DNA. The core enzyme and holoenzyme were shown to be 100% competent for binding poly[d(A-T)] in both 10 and 100 mM KCl, as assayed by nondenaturing PAGE (data not shown).

These results give direct evidence that not only the holoenzyme but also the core enzyme can open double-stranded poly[d(A-T)]. Other techniques were employed to further study the possible interaction of the core enzyme with DNA.

Salt- and Conformation-Dependent Promoter Binding. Binding of the core RNA polymerase to specific binding sites, i.e., promoters, was studied according to the protection method under a variety of salt conditions. The two promoters studied were those for the β-lactamase (P1) and the tetracycline resistance (P2) genes of pBR322 (Brosius et al., 1982). Enough polymerase was added to cover only 20% of the plasmid, but it was 3–4 times as much as needed to bind to the four major promoters of pBR322 (Stüber & Bujard, 1981). *Hin*dIII cleaves at only one site on pBR322, in a staggered cut at positions 29 and 33 (3 base pairs upstream of the P2 Pribnow box and 3–4 base pairs downstream from the P1 start site). Therefore, the *Hin*dIII cleavage site should be covered if either promoter site is occupied by RNA polymerase (Rosenberg & Court, 1979; Siebenlist et al., 1980; von Hippel et al., 1982). Cleavage of *Ava*I-linearized pBR322 with *Hin*dIII gives rise to two fragments, 2967 and 1395 base pairs long. If the polymerase blocks the cleavage by *Hin*dIII, the DNA remains a linear piece 4362 base pairs long.

The results (Figure 3) show that in the control the holoenzyme consistently blocks cleavage at the promoters, as expected from previous reports (Hsieh & Wang, 1976). The core enzyme protects the promoter region from cleavage in a salt-dependent manner, protection being greatest at a total

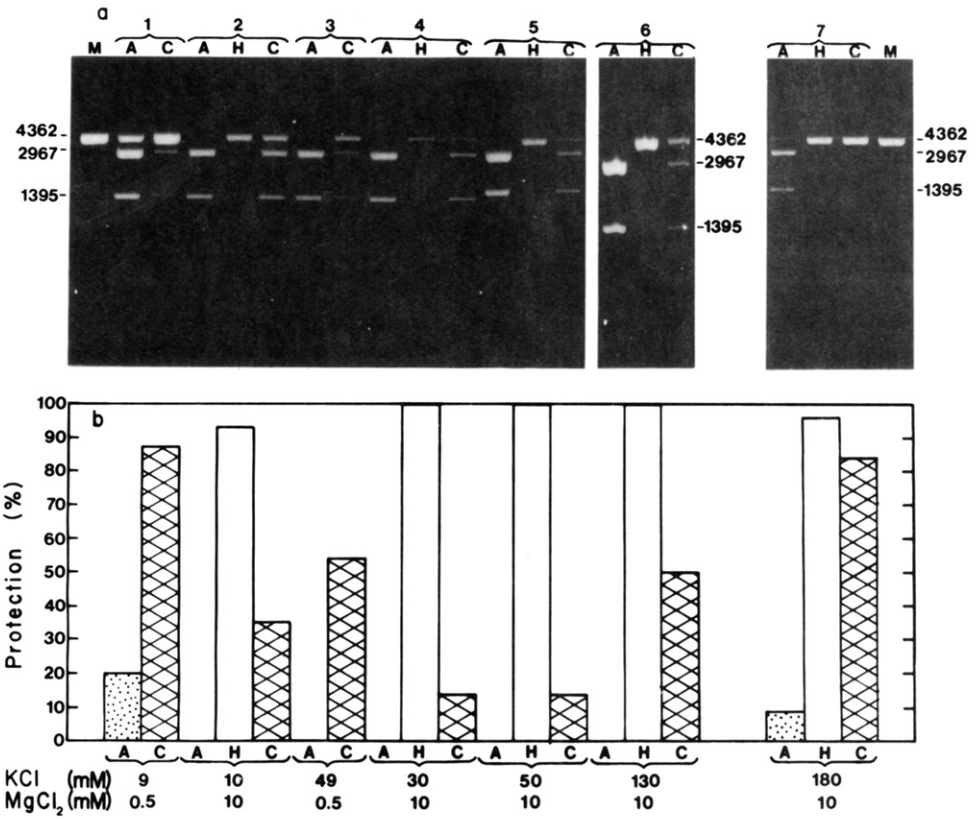

FIGURE 3: RNA polymerase protection of P1 and P2 promoters of linear pBR322 against digestion with *Hin*dIII: 40 μM *Ava*I-linearized pBR322, 150 nM polymerase, 0.1 μg/μL bovine serum albumin, and 1 mM HEPPS, pH 7.9. (a) 1% agarose gel electrophoresis in Tris–acetate of the digestion products stained with ethidium bromide. M, 4362-base-pair marker; A, no polymerase; H, with holoenzyme; C with core enzyme; group 1, 9 mM KCl, 0.5 mM $MgCl_2$; group 2, 10 mM KCl, 10 mM $MgCl_2$; group 3, 49 mM KCl, 0.5 mM $MgCl_2$; group 4, 30 mM KCl, 10 mM $MgCl_2$; group 5, 50 mM KCl, 10 mM $MgCl_2$; group 6, 130 mM KCl, 10 mM $MgCl_2$; group 7, 180 mM KCl, 10 mM $MgCl_2$. (b) Percent protection by polymerase of pBR322. The bars correspond to the lanes in (a). Dotted bars, no polymerase; open bars, holoenzyme; cross-hatched bars, core enzyme.

chloride ion concentration of 10 (including 0.5 mM $MgCl_2$) and 200 mM (including 10 mM $MgCl_2$) and decreasing at intermediate concentrations. The high promoter protection levels and the difference in the pattern of salt dependence cannot be accounted for by contamination of the core enzyme preparation with holoenzyme. We cannot detect any σ in our core preparations, based upon Coomassie blue stained or silver-stained polyacrylamide gels. Also, if the protection were due to the presence of contaminating σ, the pattern of salt-dependent protection would be the same for both the holoenzyme and core enzyme. Therefore, the protection of promoters observed here is due to the core enzyme alone, not a contamination with σ.

The location of the nonspecific binding sites of the core polymerase on linear, double-stranded DNA was also studied by using the protection technique. The core enzyme was incubated with *Hin*dIII-linearized pBR322 under a variety of salt conditions. The complexes were digested with *Hae*III, which cleaves pBR322 at many places. Enough core polymerase was used to theoretically cover the entire plasmid, but in all of the samples, regardless of the salt concentrations, digestion was complete and gave the same pattern as for *Hae*III digestion without polymerase present (data not shown).

The binding of the core to supercoiled promoters was studied by incubating the polymerase with supercoiled pBR322 and then cutting with *Hin*dIII. The relative amounts of supercoiled, relaxed-circular, and linear pBR322 resulting from this treatment indicate the efficiency of the protection of the supercoiled or relaxed-circular promoter. The results are shown in Figure 4. The holoenzyme consistently blocks cleavage at the supercoiled promoters (Figure 4b). The core enzyme, however, does not protect the supercoiled promoter region, regardless of the salt conditions, but the background indicates some nonspecific binding. The degree of protection of promoters in a relaxed-circular plasmid (Figure 4c) was assessed for comparison with the previous results (Figure 3) with linear plasmid. The holoenzyme again shows protection of the promoters under all conditions. The core enzyme protects the promoter region in a salt-dependent manner which agrees well with the results on the *Ava*I-linearized plasmid. The protection by the core polymerase is greatest at 180 mM KCl and 10 mM $MgCl_2$. The core enzyme also protects the relaxed-circular promoters well in 99 mM KCl and 0.5 mM $MgCl_2$, a condition not studied with the promoters in linear DNA. These results show that the core RNA polymerase can bind specifically to promoters. This binding of the core enzyme is very sensitive to the salt concentration. The core binding specificity is also affected by the conformation of the template. Core polymerase can bind to these promoters in linear DNA but not in supercoiled DNA.

Limited Specific Transcription. The endonuclease protection experiment demonstrated that the core enzyme binds to the region of pBR322 containing promoters P1 and P2. A limited transcription assay was used to determine whether the holoenzyme and core enzyme bound to the promoters are capable of intiating transcription and whether the initiation shows the same salt dependence as the binding. Under a variety of salt conditions, RNA polymerase was preincubated with *Pst*I-linearized pBR322 with the dinucleotide appropriate for initiation from the promoter of interest. The polymerase was allowed to transcribe from the promoter after addition of some, but not all, of the four nucleotide triphosphates, thus

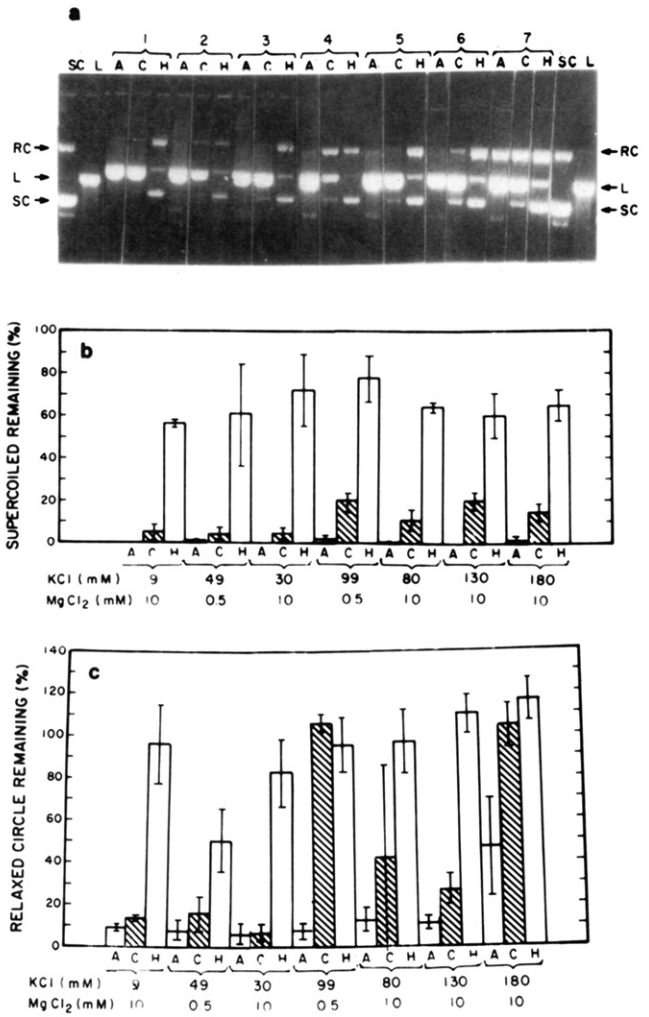

FIGURE 4: RNA polymerase protection of P1 and P2 promoters of supercoiled pBR322 against digestion with *Hin*dIII: 40 μM 70% supercoiled, 30% relaxed-circular pBR322, 75 nM polymerase, 0.1 μg/μL bovine serum albumin, and 1 mM HEPPS, pH 7.9. (a) 1% agarose gel electrophoresis in Tris–acetate of the digestion products stained with ethidium bromide. SC, supercoiled; RC, relaxed circular; L, linear; A, no polymerase; C, with core enzyme; H, with holoenzyme; group 1, 9 mM KCl, 10 mM $MgCl_2$; group 2, 49 mM KCl, 0.5 mM $MgCl_2$; group 3, 30 mM KCl, 10 mM $MgCl_2$; group 4, 99 mM KCl, 0.5 mM $MgCl_2$; group 5, 80 mM KCl, 10 mM $MgCl_2$; group 6, 130 mM KCl, 10 mM $MgCl_2$; group 7, 180 mM KCl, 10 mM $MgCl_2$. (b) Percent supercoiled pBR322 remaining = 100 (percent supercoiled after incubation per percent supercoiled without incubation). (c) Percent relaxed circular pBR 322 remaining = 100 (percent relaxed circle after incubation per percent relaxed circle without incubation). The bars correspond to the lanes in (a). Cross-hatched bars are with core enzyme. Error bars are standard deviation.

forming short, specific transcripts. Three promoters were used. The start site region of the tetracycline resistance P2 promoter is ...GGTAGTTTATCACAG... (Rosenberg & Court, 1979; Brosius et al., 1982), and that of the β-lactamase P3 promoter is ...CCCTGATAAATGC... (West & Rodriguez, 1980). These two promoters were studied in *Pst*I-linearized pBR322. The region around the start site for the M13 gene II promoter is ...CCTGTTTTTGGG... and was present on Δ229. The bases coding for the primary, limited transcripts observed in this study are underlined. The P1 promoter was not studied here because the precise start site for the P1 promoter is not known (Brosius et al., 1982). The P3 promoter was included for comparison since it has been more widely studied. The gene II promoter was used to form a hexamer (previously characterized; Osumi-Davis et al., submitted for publication) for use as a standard size marker. The limited transcripts

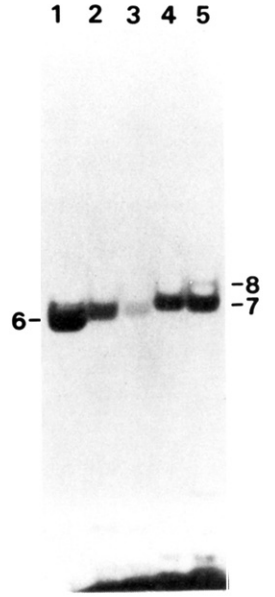

FIGURE 5: Limited transcription from promoters P2 and P3 and pBR322 by holo RNA polymerase, fractionated by urea–PAGE in Tris–borate buffer, visualized by autoradiography: 2.5 μg of holoenzyme, 130 mM NaCl, 10 mM $MgCl_2$, and 10 mM HEPPS, pH 7.9. Lane 1, hexamer marker GpUpUpUpUpU formed from transcription of M13 gene II with 0.4 μg of Δ229, 100 μM GpU, 50 μM UTP, and 17 μCi of [α-^{32}P]UTP; lane 2, hexamer GpUpUpUpApU formed by transcription promoter P2 with 8 μg of linear pBR322, 100 μg of GpU, 50 μM ATP, 50 μM UTP, and 17 μCi of [α-^{32}P]UTP; lane 3, hexamer as in lane 2 and 10-mer from promoter P2, using same as in lane 2 plus 50 μM CTP; lane 4, heptamer GpApUpApApApU formed by transcription of promoter P3 with 8 μg of linear pBR322, 100 μg of GpA, 50 μM ATP, 50 μM UTP, and 17 μCi of [α-^{32}P]ATP; lane 5, heptamer as in lane 4 and octamer GpApUpApApApUpG formed by transcription from promoter P3, using same as in lane 4 plus 50 μM GTP.

formed by the holoenzyme from the gene II, P2, and P3 promoters in 150 mM NaCl and 10 mM $MgCl_2$ (Figure 5) indicate that the holoenzyme produces the proper transcripts of the expected size. The transcripts were further characterized by their RNase A digestion patterns. The P2 hexamer and the P3 heptamer yield the expected, radioactively labeled dimer, ApU, and tetramer, ApApApU, digestion products, respectively (data not shown).

The salt dependence of the specific, limited transcription from promoter P2 to form a hexamer is shown in Figure 6. Both the holoenzyme and core enzyme show an appreciable amount of transcription under all of the conditions used. However, the salt dependence of the initiation does not reflect that of the promoter binding shown in Figures 3 and 4. Initiation by the core enzyme shows no dependence on the magnesium ion concentration; it increases under the conditions where binding is observed to be poorest and decreases where the promoter binding increases. The initiation by holoenzyme is sensitive to both the chloride and magnesium ion concentrations.

The salt dependence of the initiation from the P3 promoter to form the heptamer is shown in Figure 7. The pattern of salt-dependent initiation is different from that from the P2 promoter, especially in the case of the core enzyme, which shows a constant increase in initiation with increasing chloride ion concentration. Both the core enzyme and holoenzyme show little dependence on the magnesium ion concentration. While an endonuclease protection assay was not available for the P3 promoter, it was possible to study the binding of polymerase to this promoter in the Δ587 fragment by using a PAGE method (Fried & Crothers, 1981; Garner & Revzin, 1981).

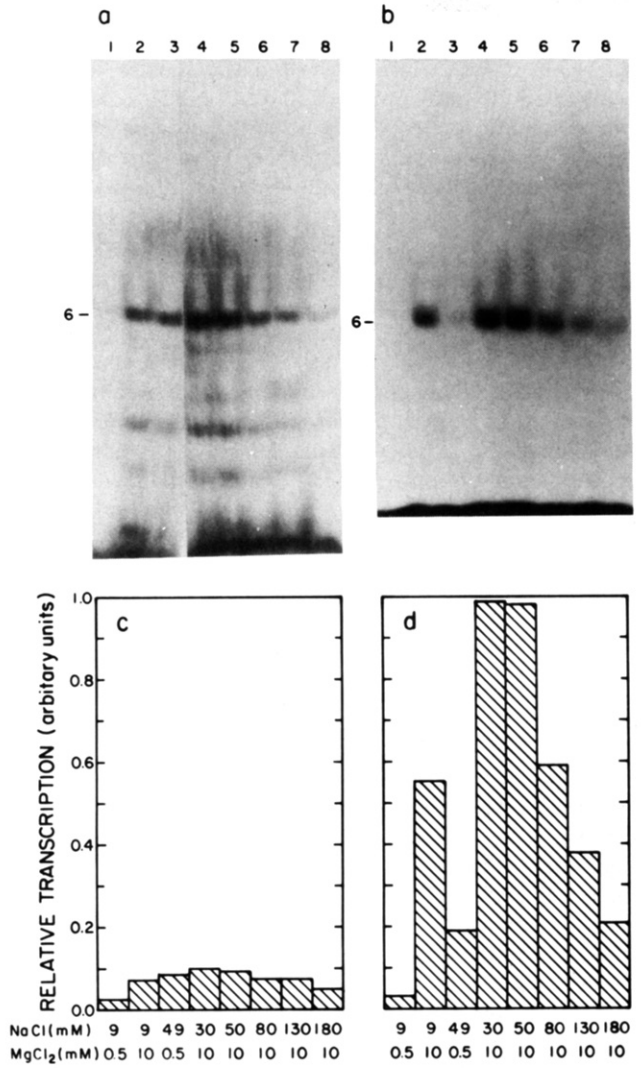

FIGURE 6: Limited transcription by RNA polymerase from promoter P2 of pBR322 to form hexamer GpUpUpUpApU, fractionated by urea–PAGE in Tris–borate buffer and visualized by autoradiography: 8 μg of *Pst*I-linearized pBR322, 100 μM GpU, 50 μM ATP, 50 μM UTP, 17 μCi of [α-^{32}P]UTP, and 10 mM HEPPS, pH 7.9. (a and b) Autoradiographs of polyacrylamide gels; (c and d) relative transcription; (a and c) with 2.4 μg of core enzyme; (b and d) with 2.5 μg of holoenzyme. Lane 1, 9 mM NaCl, 0.5 mM $MgCl_2$; lane 2, 9 mM NaCl, 10 mM $MgCl_2$; lane 3, 49 mM NaCl, 0.5 mM $MgCl_2$; lane 4, 30 mM NaCl, 10 mM $MgCl_2$; lane 5, 50 mM NaCl, 10 mM $MgCl_2$; lane 6, 80 mM NaCl, 10 mM $MgCl_2$; lane 7, 130 mM NaCl, 10 mM $MgCl_2$; lane 8, 180 mM NaCl, 10 mM $MgCl_2$.

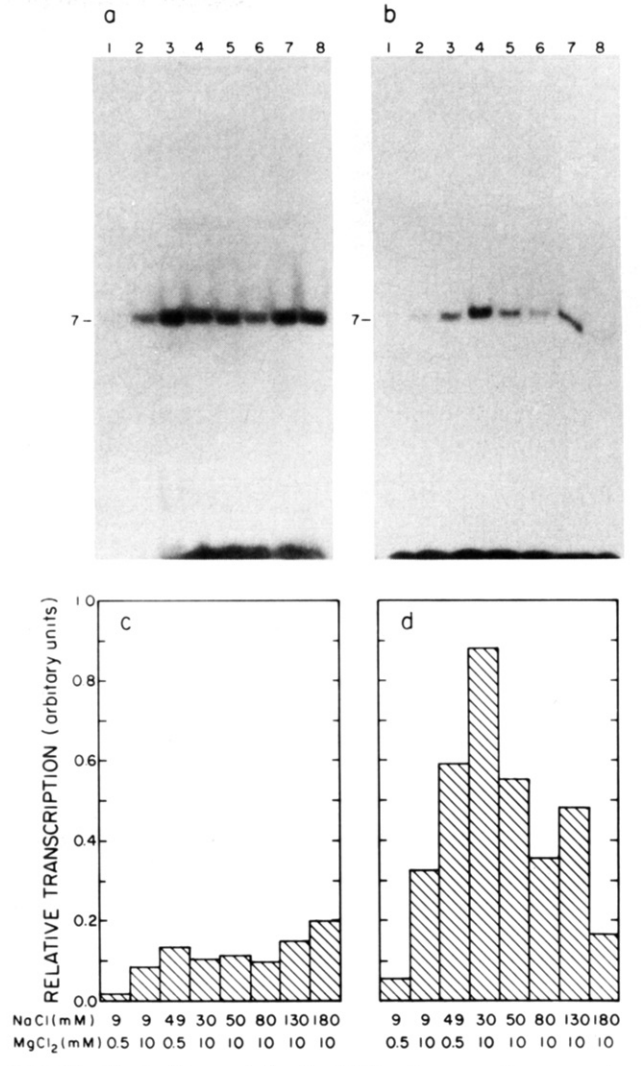

FIGURE 7: Limited transcription by RNA polymerase from promoter P3 of pBR322 to form heptamer GpApUpApApApU, fractionated by urea–PAGE in Tris–borate buffer and visualized by autoradiography: 8 μg of *Pst*I-linearized pBR322, 100 μM GpA, 50 μM ATP, 50 μM UTP, 17 μCi of [α-^{32}P]ATP, and 10 mM HEPPS, pH 7.9. (a and b) Autoradiographs of polyacrylamide gels; (c and d) relative transcription; (a and c) with 2.4 μg of core enzyme; (b and d) with 2.5 μg of holoenzyme. Lane 1, 9 mM NaCl, 0.5 mM $MgCl_2$; lane 2, 9 mM NaCl, 10 mM $MgCl_2$; lane 3, 49 mM NaCl, 0.5 mM $MgCl_2$; lane 4, 30 mM NaCl, 10 mM $MgCl_2$; lane 5, 50 mM NaCl, 10 mM $MgCl_2$; lane 6, 80 mM NaCl, 10 mM $MgCl_2$; lane 7, 130 mM NaCl, 10 mM $MgCl_2$; lane 8, 180 mM NaCl, 10 mM $MgCl_2$.

Both the holoenzyme and core enzyme bind the DNA fragment, retarding its migration into the gel in 10 mM $MgCl_2$, at both 75 and 180 mM KCl (Figure 8). When heparin (0.1 μg/μL) is added to the control, the holoenzyme remains bound to the DNA under both salt conditions, but the binding appears to be slightly weaker at the higher salt concentration since some free fragment is seen. The core enzyme does not remain bound to the promoter-containing fragment under either salt condition once heparin is added.

Except for the difference in sensitivity to magnesium, the salt dependence of initiation from the two promoters is similar for the holoenzyme, the activity being greatest at a total chloride ion concentration of 50–70 mM. The difference between the salt dependence of limited transcription by the core enzyme and the holoenzyme would not be observed if the transcription observed with the core enzyme were simply due to contaminating holoenzyme, and the relative levels of transcription are far higher than can be expected for such a contaminant in our preparation (as mentioned earlier). These results show that both the holoenzyme and core enzyme are competent for limited, specific transcription, but they differ in their dependence upon the salt concentrations and promoter sequences.

Discussion

It has been shown that the core enzyme is capable of binding double-stranded DNA even at low KCl concentrations without Mg^{2+} (Reisbig et al., 1981). We have also shown that core enzyme is transcriptionally active at this salt concentration. Difference absorption can be used to measure the opening of DNA upon polymerase binding (Hsieh & Wang, 1978; Reisbig et al., 1979; Shimer, 1985). Our results from using this technique indicate that core enzyme binding at this low salt concentration (10 mM KCl) leads to the opening of ca. 5 base pairs, while ca. 7 base pairs are opened by the holoenzyme. Opening of 10–17 base pairs has been reported for the holoenzyme at higher salt concentrations (Siebenlist, 1979; Wang

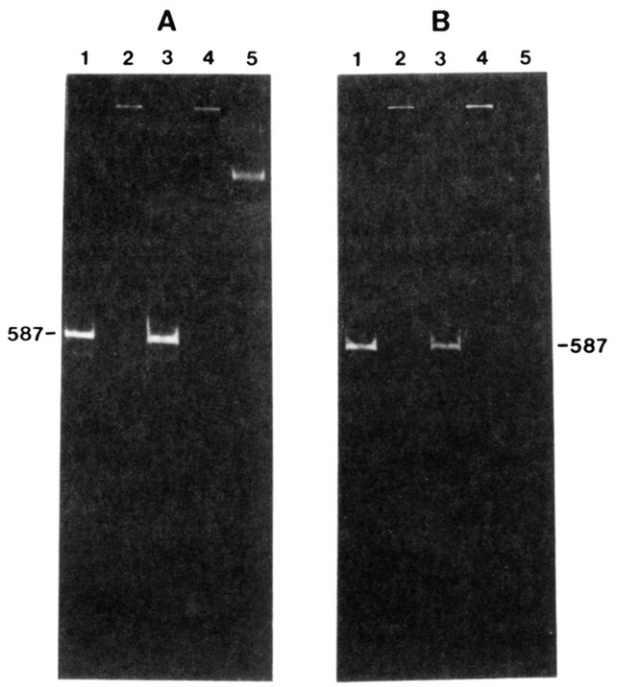

FIGURE 8: Heparin challenge of polymerase binding to the β-lactamase P3 promoter of pBR322 fractionated by 4% nondenaturing PAGE in Tris–acetate buffer, stained with ethidium bromide: 83 μM Δ587, 270 nM enzyme, and 40 mM Tris, pH 7.9. (A) 75 mM KCl, 10 mM $MgCl_2$. (B) 180 mm KCl, 10 mM $MgCl_2$. Lane 1, Δ587; lane 2, Δ587 + core enzyme; lane 3, Δ587 + core enzyme + 0.1 μg/μL heparin; lane 4, Δ587 + holoenzyme; lane 5, Δ587 + holoenzyme + 0.1 μg/μL heparin.

et al., 1977; Hsieh & Wang, 1978; Melnikova et al., 1978; Reisbig et al., 1979; Gamper & Hearst, 1982). The opening of promoters (Roe & Record, 1985) and synthetic polynucleotides (Shimer, 1985) has been reported to be dependent upon the salt concentration. The complexes of the holoenzyme and core enzyme with poly[d(A-T)] may be similar to those with promoters. While the nonspecific binding complex is believed to be closed, the complexes we observe with poly[d(A-T)] are obviously open. The −35 and −10 regions of promoters are A-T rich and may be mimicked by the alternating sequence of this synthetic DNA. The high activity of the polymerase on this template may be due to both the ease of melting A-T base pairs and the similarity of the sequence to that of promoters.

We have also found that the core enzyme is capable of specific binding to promoters. However, it appears that the conformation of the core– and holo–P3 promoter complexes are different, and the stability of the core–P3 promoter complex is lower than that of the holo–P3 promoter complex as demonstrated by its lack of heparin resistance. The core–poly[d(A-T)] complex, which is an open complex, also undergoes dissociation under the same heparin challenge, whereas the holo–poly[d(A-T)] complex remains associated (unpublished results). Heparin is able to dissociate an enzyme–promoter complex even in the open complex form, depending upon the concentration of heparin and the conformation and the stability of the enzyme–promoter complex (Pfeffer et al., 1977).

The difference between the holoenzyme and core enzyme in the salt dependence of their promoter binding may be due to the involvement of the core enzyme in more complex equilibria between DNA-bound and unbound forms. The core enzyme is much more subject to aggregation than the holoenzyme (Berg & Chamberlin, 1970; Reisbig et al., 1981; Shaner et al., 1982), forming up to octamers in 38 mM NaCl and 10 mM $MgCl_2$. Further aggregation (as measured by turbidity) occurs in the presence of double-stranded DNA below 300 mM KCl, increases with the addition of Mg^{2+}, and peaks at a KCl concentration of 50 mM (Reisbig et al., 1981). The binding of the core enzyme to the plasmid promoters shows an inverse correlation with the known patterns of both the core enzyme aggregation and aggregation with DNA. Holoenzyme, on the other hand, shows relatively little aggregation with changes in ionic strength and pH and addition of DNA (Berg & Chamberlin, 1970; Reisbig et al., 1981; Shaner et al., 1982), forming only what is interpreted as a dimer at low salt [$\mu = 0.04$, Berg and Chamberlin (1970); 38 mM NaCl, Shaner et al. (1982)]. The present results indicate that the holoenzyme binds to the promoters over the entire salt range studied, consistent with binding equilibria less complicated by salt-dependent aggregation. The inverse correlation of aggregation and promoter binding capability suggests that the aggregation of the core polymerase competes with its specific binding to DNA.

The formation of large aggregates of core enzyme with double-stranded DNA at 50 mM KCl may be due to a three-dimensional network of DNA strands and core polymerase. This could be accounted for if the polymerase has two DNA-binding sites as proposed by Reisbig et al. (1981). In the holoenzyme, σ may cover a part of the core enzyme DNA-binding site necessary for binding a second polynucleotide (Reisbig et al., 1981). This is supported by the observation that core enzyme releases twice as many sodium ions as the holoenzyme upon binding double-stranded DNA, suggesting a larger DNA binding site on core polymerase (deHaseth et al., 1978). The effect of salt on aggregation may partially mimic the effect of σ binding, shifting the predominant form of the core from one able to form a nonproductive complex with DNA to one that forms a productive complex with the promoter. Given that the polymerase and DNA conformations are both sensitive to ionic strength, it is not surprising that the transition between an inactive polymerase–DNA complex and an active, preinitiation complex is a salt-dependent equilibrium that varies from template to template (Domingo et al., 1975b; Roe & Record, 1985; this work).

The binding of σ must, however, do more than simply favor the same conformation as is obtained by altering the salt concentration, for the σ subunit allows the polymerase to bind to the P1 and P2 promoters in supercoiled as well as linear DNA. The ability of σ to alter the sensitivity of polymerase to DNA conformation may come from a unique σ–core enzyme interaction. This is supported by the work of Wu et al. (1976), which indicates that there is a conformational transition of RNA polymerase induced by the interaction of σ with core enzyme.

Transcriptional activity is very sensitive to ionic strength in a manner dependent upon the template studied (Küpper et al., 1976; Stevens, 1976; Khesin et al., 1976). This sensitivity is at the level of initiation (Domingo et al., 1975b; McClure et al., 1978). In part, this must be due to binding. However, direct comparison between promoter binding and initiation must be made with caution. The best conditions for binding and initiation are not always the same (McClure et al., 1978; Ishihama, 1986; this work). Promoters with a high binding rate constant but low affinity can be more efficient at initiation than promoters of high affinity but with slow on and off rates (Seeburg et al., 1977). Therefore, the salt dependence of the early steps of transcription must be studied, as well as the promoter binding of the core enzyme and holoenzyme.

The core polymerase is transcriptionally active without the σ subunit, retaining roughly 50% of the activity of the holoenzyme on calf thymus DNA. All core enzyme activity has generally been attributed to nonspecific transcription initiated at nicks or ends (Chamberlin, 1976), despite the observations that (1) initiation at nicks and free ends of the DNA cannot account for all of the RNA synthesis by the core enzyme (Vogt, 1969); (2) initiation by core, generally proposed to be at nicks, is actually sequence dependent (Dausse et al., 1972); and (3) only holoenzyme shows an affinity for ends and neither holoenzyme nor core enzyme shows a preference for nicks (Melançon et al., 1983). Specific transcription by the core enzyme may have been overlooked since assays relying on asymmetry of transcription from a total genome are often not sensitive enough to detect specific transcription (Kassavetis et al., 1983). The core enzyme of *Azotobacter vinelandii* RNA polymerase has been shown to transcribe S13RF DNA specifically (Domingo et al., 1975a). This enzyme shows transcriptional activity and specificity similar to that of the *E. coli* enzyme; its σ subunit associates with *E. coli* σ (Domingo et al., 1975a). We show here that *E. coli* core polymerase can also initiate specific transcription. The efficiency of the limited transcription is promoter dependent and varies with the salt conditions used in a manner different from the promoter binding. The maximal efficiency of the holoenzyme-limited transcription is 5 times higher than that of the core enzyme for a given promoter. However, the core transcription can reach an efficiency equal to that of the holoenzyme under some salt conditions. While these salt-dependent differences of initiation efficiency between the holoenzyme and core enzyme may reflect differences in their surface charge distributions, the exact pattern is promoter specific and must be due to either the DNA or RNA sequence. The difference in the relative, maximal transcription by the two forms of enzyme also goes beyond any ionic interaction and must be due to a specific interaction of σ with the core enzyme in the complex with the DNA and growing RNA chain.

ACKNOWLEDGMENTS

We thank Patricia Osumi-Davis for the preparation of the 229-base-pair fragment of pM13/AI25. We are grateful to Dr. Paul Hagerman, University of Colorado, Denver, for the kind gift of the pM13/AI25 plasmid containing gene II of phage M13. We also thank Dr. James Bamburg for allowing us to use his Beckman DU-8B spectrophotometer with gel-scanning attachment.

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Guanosine Binding Required for Cyclization of the Self-Splicing Intervening Sequence Ribonucleic Acid from *Tetrahymena thermophila*†

N. Kyle Tanner‡ and Thomas R. Cech*
Department of Chemistry and Biochemistry, University of Colorado, Boulder, Colorado 80309-0215
Received October 16, 1986; Revised Manuscript Received January 22, 1987

ABSTRACT: We have converted the intramolecular cyclization reaction of the self-splicing intervening sequence (IVS) ribonucleic acid (RNA) from *Tetrahymena thermophila* into an intermolecular guanosine addition reaction. This was accomplished by selectively removing the 3′-terminal nucleotide by oxidation and β-elimination; the β-eliminated IVS thereby is no longer capable of reacting with itself. However, under cyclization conditions, a free guanosine molecule can make a nucleophilic attack at the normal cyclization site. We have used this guanosine addition reaction as a model system for a Michaelis–Menten kinetic analysis of the guanosine binding site involved in cyclization. The results indicate that functional groups on the guanine that are used in a G-C Watson–Crick base pair are important for the cyclization reaction. This is the same result that was obtained for the guanosine binding site involved in splicing [Bass, B. L., & Cech, T. R. (1984) *Nature (London) 308*, 820–826]. Unlike splicing, however, certain additional nucleotides 5′ to the guanosine moiety make significant binding contributions. We conclude that the guanosine binding site in cyclization is similar to, but not identical with, the guanosine binding site in splicing. The same binding interactions used in cyclization could help align the 3′ splice site of the rRNA precursor for exon ligation. We also report that the phosphodiester bond at the cyclization site is susceptible to a pH-dependent hydrolysis reaction; the phosphodiester bond is somehow activated toward attack by the 3′-hydroxyl of a guanosine molecule or by a hydroxyl ion.

The 26S rRNA precursor of the ciliated protozoan *Tetrahymena thermophila* contains an intervening sequence (IVS)[1] that has intramolecular catalytic activity. It is capable of excising itself from the precursor RNA, in vitro, in the absence of any protein or external energy source (Kruger et al., 1982). The reaction is initiated by the nucleophilic attack of the 3′-hydroxyl of a guanosine cofactor at the phosphodiester bond at the 5′ splice site. The 5′ exon is released, and the guanosine cofactor is covalently attached to the 5′ end of the IVS (Figure 1, step a). The free 3′-hydroxyl of the 5′ exon can then make a nucleophilic attack at the phosphodiester bond at the 3′ splice site to release the IVS and ligate together the exons (Figure 1, step a′). It is now known that this reaction is not unique to the *Tetrahymena* pre-rRNA; group I introns from other organisms are capable of a similar, self-catalyzed splicing reaction (Garriga & Lambowitz, 1984; Van der Horst & Tabak, 1985; Chu et al., 1986).

The released IVS RNA retains catalytic activity. The 3′-hydroxyl of the guanosine nucleotide at the 3′ end of the IVS can make a nucleophilic attack at the phosphodiester bond between nucleotides 15 and 16, release a 15-nucleotide fragment (15-mer) containing the nonencoded guanosine, and form a covalently closed circle (Figure 1, step b). A minor product is formed by nucleophilic attack between nucleotides 19 and 20 to form a slightly smaller circle and release a 19-nucleotide fragment (19-mer). These cyclization reactions share many features with splicing. All are transesterification reactions where the total number of phosphodiester bonds is conserved

† Supported by the American Cancer Society (NP-374C).
* Correspondence should be addressed to this author. He is an American Cancer Society Research Professor.
‡ Present address: Division of Biology, California Institute of Technology, Pasadena, CA 91125.

[1] Abbreviations: RNA, ribonucleic acid; IVS, intervening sequence; pre-rRNA, ribosomal RNA precursor; L - 14 IVS, linear IVS RNA with the first 15 nucleotides replaced by guanosine or by a guanosine analogue; L - 15 IVS, linear IVS RNA produced from site-specific hydrolysis at the cyclization site of either the circular IVS or the linear IVS; EDTA, ethylenediaminetetraacetic acid; G, guanosine; HEPPS, *N*-(2-hydroxyethyl)piperazine-*N*′-3-propanesulfonic acid; Tris, tris(hydroxymethyl)-aminomethane; 15-mer, an RNA fragment released during the cyclization reaction that contains the first 15 nucleotides of the linear IVS; 19-mer, a 19-nucleotide RNA fragment released during cyclization at a minor site; L IVS_β, linear IVS RNA with its 3′-terminal guanosine removed by periodate oxidation and β-elimination; HPLC, high-performance liquid chromatography; TLC, thin-layer chromatography.

0006-2960/87/0426-3330$01.50/0