THE BINDING SITES FOR *ESCHERICHIA COLI* RNA POLYMERASE ON λ PHAGE DNA: CHARACTERISTICS OF BINDING SITES ON SONICATED λ DNA

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1. Introduction

RNA polymerase can bind various polynucleotides of low molecular weight such as synthetic polynucleotides [1, 2] or tRNA [3]. Denatured or badly damaged DNA binds the polymerase non-specifically [4]. These observations suggest that the specificity of binding between DNA template and RNA polymerase depends much on the structure of the template. However, it was indicated by Cohen, Maitra and Hurwitz [5] that the regions of λ DNA transcribed in vitro were not significantly changed by shearing or open-circular formation of the template. Since 'binding' and 'initiation' by RNA polymerase are separately controlled processes [6], it is of interest to examine whether structural alterations in DNA result in changes of binding specificity. In the present paper we report the characteristics of binding of RNA polymerase to λ DNA which had been fragmented by sonication.

2. Materials and methods

RNA polymerase was prepared from *E. coli* B(H) cells as described previously [7]. ^{33}P-Phosphoric acid was purchased from the New England Nuclear Corp., Boston, Mass., USA. ^{33}P-labelled λ DNA was prepared by phenol extraction [8]. λ DNA in 0.01 M tris-HCl buffer (pH 7.0) containing 0.2 M NaCl was sonicated at 10 kHz (50–110 W) for 5–10 min under N_2 atmosphere. Sonicated solution was mixed with an equal amount of 0.1 M acetate buffer (pH 5.0) and treated with nuclease S_1 (200 units/ml) at 37° for 1 hr to remove single-stranded regions [9] which may have been produced by sonication. The fragment of λ DNA was obtained as a homogeneous peak by glycerol gradient centrifugation, leaving less than 5% of smaller fragments near the meniscus.

3. Results

The DNA–RNA polymerase complex can be retained on a nitrocellulose filter, while uncomplexed DNA and RNA polymerase pass through the filter [10]. Using this technique, we examined the complex formation of sonicated λ DNA with the polymerase. Fixed amounts of sonicated λ DNA were mixed with increasing amounts of RNA polymerase and the amounts of DNA retained on filters were measured. The results shown in fig. 1 indicate that the preparations of λ DNA fragments with average molecular weights of 3×10^6 daltons or 5.5×10^6 daltons give maximum retention – 2–3 units of RNA polymerase per μg DNA. This amount of polymerase is almost the same as that obtained using intact λ DNA [7]. In contrast, no obvious saturation point was observed with λ DNA fragments of about 1×10^6 daltons.

Pettijohn and Kamiya [12] reported that RNA polymerase binds non-specifically to polyoma DNA at low ionic strength. Although intact λ DNA shows specific binding with RNA polymerase in the binding buffer used (tris-HCl, 40 mM, pH 8.0; $MgCl_2$, 8 mM) without addition of salt [11], the low molecular weight of polyoma DNA (3×10^6 daltons) suggests that highly fragmented λ DNA may require high ionic strength for its specific binding. To confirm this, we re-examined the retention experiment of the fragment of 1×10^6 daltons, with the addition of 0.1 M KCl in

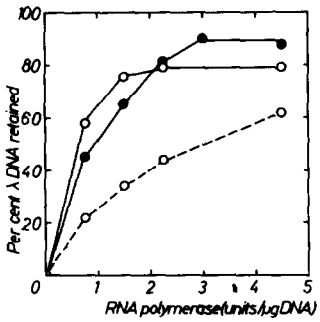

Fig. 1. Retention of the λ DNA fragment on a nitrocellulose filter with increasing amounts of RNA polymerase. The reaction mixture contained, in a final volume of 0.18 ml, 40 mM tris-HCl (pH 8.0), 8 mM $MgCl_2$, 0.2 μg ^{33}P-labelled λ DNA, and increasing amounts of RNA polymerase. After 5 min at 37°, the amount of ^{33}P-labelled λ DNA retained on a Millipore filter was determined. Average molecular weights of the λ DNA fragment used are 5.5 × 10^6 daltons (○—○), 3 × 10^6 daltons (●—●), and 1 × 10^6 daltons (○ - - - ○).

the buffer. As shown in fig. 2, a clear saturation curve was obtained. The amount of λ DNA retained on the filter did not exceed about 50% of total DNA. This suggests that about half of the 32 pieces of fragments derived from one molecule of DNA (32 × 10^6 daltons) cannot form complexes with RNA polymerase. The amount of RNA polymerase needed for the maximum retention was 3 units per μg DNA, the same as that obtained using intact λ DNA. These results suggest that in the presence of 0.1 M KCl, a λ DNA fragment of about 1 × 10^6 daltons binds specifically with RNA polymerase.

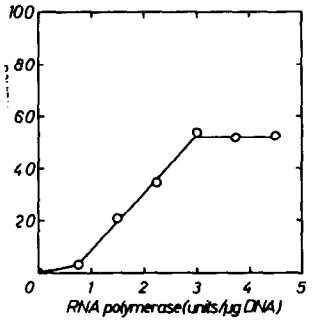

Fig. 2. Retention of the λ DNA fragment with an average molecular weight of 1 × 10^6 daltons in the presence of 0.1 M KCl. Experimental conditions were the same as in fig. 1 except that 0.1 M KCl was added to the reaction mixture.

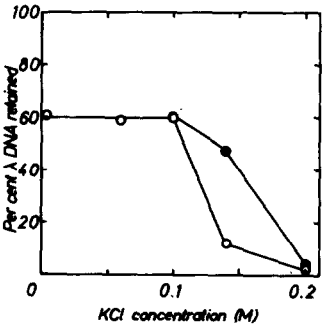

Fig. 3. Effect of KCl concentration on complex formation. The reaction mixture contained, in a final volume of 0.153 ml, 40 mM tris-HCl (pH 8.0), 8 mM $MgCl_2$, 0.11 μg ^{3}H-labelled λ DNA (average molecular weight, 1.6 × 10^6 daltons), 0.33 (○—○) or 0.66 (●—●) units of RNA polymerase, and KCl as indicated. After 5 min at 37°, the amount of ^{3}H-labelled λ DNA retained on a Millipore filter was determined.

Since the λ DNA fragments with average molecular weights of 5.5 × 10^6 or 3 × 10^6 daltons are retained almost completely in the presence of a sufficient amount of RNA polymerase (fig. 1), it is not clear whether these fragments can attach more polymerase after they have been complexed with the minimum amount of RNA polymerase needed for the maximum retention (2–3 units/μg DNA). To investigate this, it was considered important to determine the portion of DNA covered by the polymerase in the DNA–RNA polymerase complex. This amount can be measured using the filter retention technique after treatment of

Table 1
The amount of λ DNA fragment–RNA polymerase complex retained on a nitrocellulose filter after treatment with nucleases.

MW λ DNA fragment (daltons × 10^{-6})	RNA polymerase added (units/μg DNA)	λ DNA retained (%)
5.5	2.6	1.1
5.5	3.9	0.9
1	4.1	1.2

The fragment of ^{33}P-labelled λ DNA was mixed with RNA polymerase under the same conditions as in fig. 1 (5.5 × 10^6 daltons) or fig. 2 (1 × 10^6 daltons). The mixture was treated with pancreatic DNase I (300 μg/ml) and venom phosphodiesterase (80 μg/ml) at 37° for 2.5 hr. The nuclease-resistant portion of λ DNA was measured by the retention on a Millipore filter.

the complex with a high concentration of nucleases [11]. Table 1 shows that the fragment of 5.5×10^6 daltons mixed with 2.6 or 3.9 units of RNA polymerase per μg DNA gives the same nuclease-resistant portion (about 1.0%). This indicates that λ DNA fragments cease to attach the polymerase when the retention curve shown in fig. 1 reaches a plateau. Table 1 also shows that the nuclease-resistant portion in the fragment of 1×10^6 daltons mixed with 4.1 units of RNA polymerase per μg DNA is 1.2%. Previous experiments in our laboratory [7, 11] revealed that when intact λ DNA (32×10^6 daltons) was mixed with sufficient RNA polymerase, 0.9 to 1.1% of the DNA portion became resistant to nucleases. These results indicate that the number of binding sites for RNA polymerase on λ DNA is not changed by the fragmentation of DNA under the conditions tested. It can therefore be concluded that fragmentation of λ DNA to about 1×10^6 daltons has no essential effect on the specificity of binding with RNA polymerase; if a suitable salt concentration in the binding mixture is selected, the fragment binds specifically with the polymerase in the same way as intact λ DNA.

The effect of salt concentration on the DNA–RNA polymerase complex formation was further studied using a λ DNA fragment of about 1.6×10^6 daltons. Fig. 3 shows that the λ DNA fragment mixed with 3 units of RNA polymerase per μg DNA forms constant amounts of complex at KCl concentrations between 0.004 and 0.1 M, while the retention curve falls sharply at KCl concentrations greater than 0.1 M. However, the amount of retention in the mixture containing 6 units of RNA polymerase per μg DNA is not significantly reduced by 0.14 M KCl. These results led us to examine another experiment in which fixed amounts of λ DNA fragment were mixed with increasing amounts of RNA polymerase in the presence of 0.1 or 0.14 M KCl and the amounts of DNA retained on filters were measured. The results obtained revealed that while the maximum amount of λ DNA fragment retained (about 60%) was the same at both salt concentrations, the amount of RNA polymerase needed for the maximum retention at 0.14 M KCl was more than twice (about 7 units/μg DNA) that needed at 0.1 M. These results indicate that the number of binding sites is not changed by the presence of a high salt concentration, and suggest that the reduced amount of complex formation observed at high salt concentration is due to an increased equilibrium dissociation constant for the DNA–RNA polymerase interaction.

4. Discussion

The mechanism of recognition of binding sites on λ DNA by *E. coli* RNA polymerase has been studied in our laboratory. DNA regions which constitute the binding sites have been isolated and characterized [7, 11]. The number of binding sites on a λ DNA molecule was estimated to be 14 [11]. Since it has been reported that only the right half of a λ DNA molecule is transcribed in vivo by *E. coli* RNA polymerase [13], it is of considerable interest to determine the position of these sites on a DNA molecule. The data presented in this paper reveal that the specificity of binding between λ DNA and RNA polymerase remained unchanged after the DNA was sheared by sonication, followed by the treatment with nuclease S_1, into fragments with an average molecular weight of about 1×10^6 daltons. In addition, about half this fragment was retained on the filter as a complex with DNA polymerase. These results suggest that it would be possible to isolate the DNA fragments carrying only one or two binding site(s) and to determine the positions on a λ DNA molecule where these fragments were located using the DNA–DNA hybridization technique.

If the binding sites on a λ DNA molecule were in clusters, then shearing a DNA molecule into several fragments would reduce the amount of DNA retained on the filter, due to the loss of fragments lacking binding sites. However, nearly quantitative retention was observed when λ DNA sheared into about 11 pieces (about 3×10^6 daltons) was mixed with sufficient RNA polymerase, while the λ DNA sheared into about 32 pieces (fragments of 1×10^6 daltons) was retained only in part (fig. 1). These results suggest that the 14 binding sites are distributed throughout a λ DNA molecule. The question arises as to what is the relationship between the 14 binding sites and the transcription of only the right half of a λ DNA molecule observed in vivo [13]. Preliminary experiments in our laboratory indicate that only a few of the small λ DNA fragments (1×10^6 daltons) bearing binding site(s) could initiate RNA synthesis. There-

fore, it may be expected that at least two species (active and inactive) of binding sites on λ DNA exist. Isolation of the binding sites capable of RNA synthesis is in progress.

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