BINDING OF THE TERMINATION FACTOR ρ TO DNA

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Received March 31, 1971

SUMMARY

The termination factor of transcription, ρ , isolated from Escherichia coli, was found to bind to DNA. Complex formation of ρ with different polynucleotides was studied by means of retention on nitrocellulose filters. Native Escherichia coli DNA binds to ρ reversibly being replaced by other native or denatured DNA's. Actinomycin-D, does not interfere with the attachment of the termination factor to native DNA. Binding of ρ to native DNA, confers partial protection against pancreatic DNase digestion. Evidence is presented for the existence of discrete sites for the attachment of the termination factor to the DNA.

The process of RNA transcription by DNA-dependent RNA polymerase from E. coli requires initiation and termination at specific sites on the DNA template. Transcriptionally correct chain initiation is mediated by the protein factor, σ found associated with RNA polymerase (1). Recently, a new protein factor, ρ , isolated and purified by Roberts (2) from E. coli, was found to cause specific termination and release of RNA during in vitro transcription of λ DNA. The RNA products which are synthesized in the presence of the termination factor, ρ, are smaller in length (3, 4) and are transcripts of more limited regions of the DNA template (2, 5) than those synthesized in its absence. There is little information, however, about the molecular mechanism of termination mediated by this factor. Using the nitrocellulose filter technique, we have previously shown that ρ binds to DNA (6). This observation has recently been confirmed in other laboratories (7, 8). In the present communication the specificity of the interaction of ρ to DNA is examined.

MATERIALS AND METHODS

E. coli DNA was prepared as previously described (9). [^{32}P]-labeled E. coli DNA was prepared from cells grown on Tris-glucose medium (10) containing 10^{-4} M phosphate and 10-15 mC [^{32}P]-orthophosphate per liter of culture. Ø80 DNA was prepared from purified phage by phenol extraction as described previously (3). Calf thymus DNA was purchased from Sigma Chemical Co.

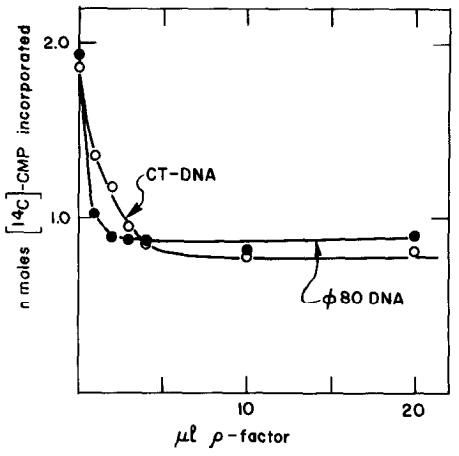

Figure 1. Effect of ρ on Ø80 and calf thymus DNA transcription. The reaction mixture contained, in a final volume of 0.1 ml: 5μmoles of Tris-HCl buffer, pH 7.9; 1 μmole of $MgCl_2$; 10 μmoles of KCl; 0.1 μmole of dithiotreitol; 0.05 μmole each of ATP, GTP, and UTP; 0.05 μmole of [^{14}C]-CTP (2.1×10^6 cpm/μmole); 5 μg of calf thymus or Ø80 DNA; 7 units of RNA polymerase (spec. act. 3000 units/mg protein) and different amounts of ρ factor as specified. The reaction mixture was incubated for 30 min at 37° followed by the addition of one drop of a bovine serum albumin solution (2 mg/ml) and cold 5% trichloroacetic acid. The precipitate was collected on Millipore filters, washed with cold trichloroacetic acid and counted in a liquid scintillation spectrophotometer.

Termination factor ρ was prepared from E. coli MRE 600 cells by following the entire procedure of Roberts (2). The activity of ρ was assayed by its ability to depress the in vitro transcription of Ø80 DNA or calf thymus DNA to a plateau value (Fig. 1).

The DNA binding assay involved filtration of termination factor-DNA complexes through nitrocellulose filters using similar conditions to those devised by Jones and Berg for the binding of RNA polymerase to DNA (11). The appropriate amounts of DNA and ρ were incubated for 5 min at 37° in a solution containing 40 mM Tris-HCl buffer, pH 7.9; 4 mM $MgCl_2$ and 12 mM β-mercaptoethanol in a total volume of 0.25 ml. After incubation, the mixture was diluted with 2.0 ml of ice-cold 10 mM Tris-HCl buffer, pH 7.9 and 50 mM NaCl (dilution buffer). The mixture was loaded with gentle suction on a Millipore filter (type HA, 0.45 μ, 25 mm), washed with 50 ml of the same buffer solution, dried and counted in a liquid scintillation spectrophotometer.

RESULTS

Binding of termination factor to DNA. The retention of native DNA on membrane filters in the presence of ρ was employed to measure the interaction between the two components. Free, native DNA washes through a Millipore membrane; however, in the presence of ρ the DNA is retained by the filter. The amount of native E. coli DNA retained is pro-

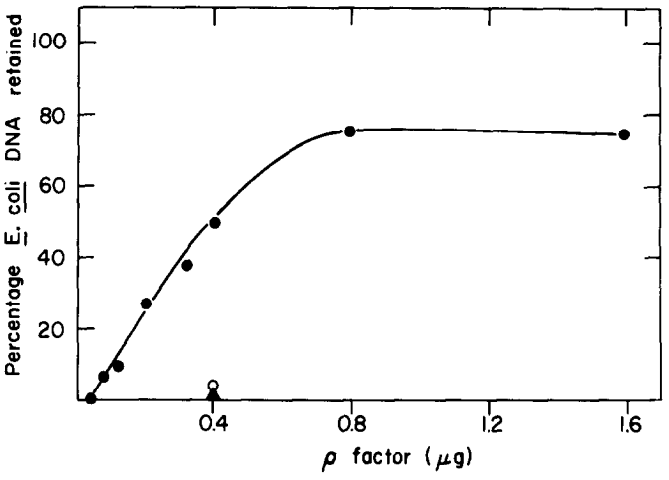

Figure 2. ρ -dependent retention of [^{32}P] -labeled E. coli DNA on membrane filters. Reaction mixtures in a final volume of 0.25 ml contained: 40 mM Tris-HCl buffer, pH 7.9; 4 mM $MgCl_2$; 12 mM β-mercaptoethanol and 2 μg of native [^{32}P] -labeled E. coli DNA ($5x10^4$ cpm/μg). After incubation for 5 min at 37° the mixture was diluted and loaded on Millipore filters as described in Materials and Methods. The percentage of DNA bound is calculated from the input [^{32}P] -labeled E. coli DNA which is set equal to 100%. A value of 2140 cpm representing binding in the absence of protein was substracted from each value.

▲ ρ factor in standard buffer solution was denatured by heating at 80° for 5 min.

0 After incubation of labeled E. coli DNA with ρ factor as described above, the mixture was made 0.4% in sodium dodecyl sulfate, kept for 3 min at 37°, diluted and filtered.

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portional to the quantity of ρ added until a plateau value is reached where about 80% of the DNA input is retained (Fig. 2). If ρ is passed through the filter first,followed by [^{32}P]-DNA, there is less than 2% retention of the labeled DNA. This value is similar to that obtained when free [^{32}P]-DNA is filtered alone. The results suggest that ρ and DNA must be incubated together in order to obtain a complex which is retained on the filter. The ability of ρ to bind to DNA is thermosensitive. After heating ρ for 5 min at 80° in the standard-buffer solution prior to incubation with native [^{32}P]-DNA less than 2% of the label is retained on the filter. Upon the addition of sodium dodecyl sulfate to a final concentration of 0.4% the ρ -DNA complex is dissociated and almost all of the DNA becomes filtrable (Fig. 2). The amount of labeled DNA in complex with ρ which is retained on the filter is not affected by concentrations of Mg^{2+} less than $8x10^{-3}$ M. Concentrations of Mg^{2+} higher than 10^{-2} M show an inhibitory effect on the binding reaction (at $2x10^{-2}$ M and $4x10^{-2}$ M only 58% and 40% of the amount bound at lower concentrations of Mg^{2+} are retained on the filter). Significant binding is also observed in the presence of 1 mM EDTA (60% of the amount bound at low Mg^{2+} concentrations).

The ρ-DNA complex is dissociated by high-salt concentration. While maximum binding is observed at NaCl concentrations below 0.05 M, the amount of [^{32}P]-labeled E. coli DNA bound on filters decreases to 60% and 30% upon dilution of the preformed complex in dilution-buffer containing 0.2 M and 0.4 M NaCl respectively. In addition, it was found that complex formation can take place rapidly at 0°. Actinomycin D was not found to interfere with complex formation between E. coli DNA and ρ at concentrations from 3×10^{-7} to 1.44×10^{-4} M. The transcription of the same amount of native DNA was inhibited over 90% by 2×10^{-6} M actinomycin D.

Denatured [^{32}P]-labeled E. coli DNA was also observed to bind to filters upon incubation with ρ. However, higher concentrations of ρ are required in order to obtain the same level of retention on the filter as found with native DNA. It should be noted that under similar conditions the E. coli initiation factor, σ, is unable to bind to native DNA. (When 2 µg of native [^{32}P]-labeled DNA (6×10^4 cpm/µg) were interacted with 2.5 µg of σ factor, less than 6% of the label was retained on the filter.)

Dissociation of the ρ-DNA complex. Dissociation of the ρ-DNA complex was studied by measuring the ability of native or denatured DNA to displace native [^{32}P]-labeled E. coli DNA from its complex with ρ. The results presented in Table I show that complex formation is a reversible reaction; [^{32}P]-labeled E. coli DNA is displaced from the

Table I. Displacement of [^{32}P]-labeled E.coli DNA from a complex with ρ by unlabeled E. coli DNA

E. coli DNA added at		Percentage [^{32}P]-DNA bound
0 min	5 min	
native [^{32}P]-DNA (2µg)	-	100
"	native DNA 2 µg	48
"	" 4	39
"	10	21
"	denatured DNA 2 µg	23
"	" 4	21
"	" 10	15

Reaction mixtures in a final volume of 0.25 ml contained: 40 mM Tris-HCl buffer, pH 7.9; 4 mM $MgCl_2$; 12 mM β-mercaptoethanol; 0.4 µg of ρ and 2 µg of [^{32}P]-labeled E. coli DNA (2.5×10^4 cpm/µg). After 5 min incubation at 37° different concentrations of unlabeled native or denatured E. coli DNA were added, kept for an additional 5 min at 37° and each mixture was filtered as described in Materials and Methods.

complex by unlabeled native E. coli DNA. Denatured E. coli DNA is more efficient in this displacement reaction.

The capacity of different nucleic acids to bind to ρ is shown in Fig. 3. In this experiment increasing amounts of several nucleic acids were incubated for 5 min with 0.4 μg of ρ, before the addition of 2 μg of native [^{32}P]-labeled E. coli DNA. Incubation was continued for an additional 5 min and the extent of label retained on the filter was then measured. The ability of native E. coli DNA to dissociate the complex between ρ and various nucleic acids depends on the nature and source of the polynucleotide used. As expected, the complex of ρ and native E. coli DNA can be readily dissociated by the addition of native [^{32}P]-labeled E. coli DNA; the complex of ρ with native calf thymus DNA was far less stable. (Incubation of a preformed complex of ρ and 4 μg of calf thymus DNA with 2 μg of [^{32}P]-labeled E. coli DNA results in displacement of calf thymus DNA, 90% of the label being retained on the filter.) On the other hand, denatured E. coli DNA as well as denatured calf thymus DNA are tenaciously bound to ρ. (Incubation of a preformed complex of ρ and 4 μg of denatured E. coli DNA with 2 μg of [^{32}P]-labeled E. coli DNA results in only 3% of the label being retained on the filter.)

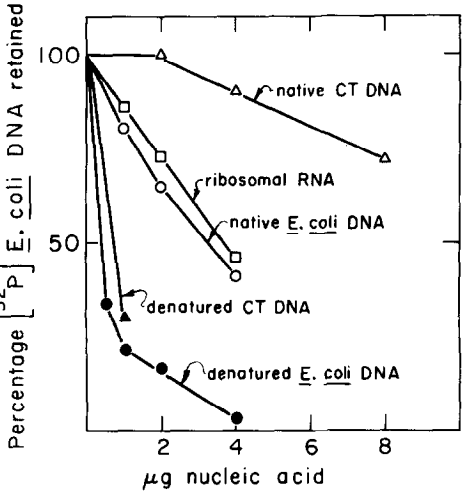

Figure 3. Prevention of complex formation between ρ and [^{32}P]-labeled E. coli DNA by prior incubation with varying concentrations of other nucleic acids. Reaction mixtures in a final volume of 0.25 ml contained: 40 mM Tris-HCl buffer, pH 7.5; 4 mM $MgCl_2$; 12 mM β-mercaptoethanol; 0.4 μg of ρ and increasing concentrations of one of the unlabeled nucleic acids. After incubating for 5 min at 37°, 2 μg of [^{32}P]-labeled E. coli DNA (4×10^4 cpm/μg) was added to each tube. Incubation was then continued for an additional 5 min and the solution diluted and loaded onto filters as described in Materials and Methods. The reaction mixture containing only ρ factor and [^{32}P]-labeled E. coli DNA was taken as representing 100% binding.

Protection from DNase degradation by complex formation with ρ. The protection conferred to DNA by complex formation with ρ against attack by pancreatic DNase was studied. The ρ-DNA complex formed between 7.8 µg of [^{32}P]-labeled E. coli DNA and 20 µg of ρ was loaded on filters, monitored for radioactivity and then incubated with 100 µg of pancreatic DNase. The results presented in experiment I of table II show that a considerable amount of the labeled DNA attached to the filters is resistant to DNase digestion. In experiment II, filters containing ρ-DNA complexes formed between 2 µg of [^{32}P]-labeled E. coli DNA and different amounts of ρ (viz., 2.5, 5 and 10 µg) were treated with 50 µg of pancreatic DNase. It can be seen that addition of increasing quantities of ρ increases the amount of labeled DNA resistant to attack by DNase up to a plateau value.

Table II. Protection from DNase digestion by complex formation with ρ.

Experiment	Additions	Radioactivity on filter	
		Before DNase	After DNase
		(cpm)	(cpm)
I [^{32}P] - DNA(7.8 µg)	-	1,300	1,100
"	10 µg BSA	1,600	1,400
"	20 µg ρ	115,000	14,000
II [^{32}P]- DNA (2 µg)	-	-	500
"	2 µg BSA	-	700
"	2.5 µg ρ	-	2,300
"	5 µg ρ	-	4,200
"	10 µg ρ	-	3,800

After incubation of native [^{32}P]-labeled E. coli DNA (input 145,000 cpm) with different amounts of ρ or bovine serum albumin, the samples were loaded onto membrane filters as described in Materials and Methods. The wet, washed filters were counted in a gas flow counter and then immersed in 300 µl of a buffer containing 10 mM Tris-HCl, pH 7.5; 10 mM $MgCl_2$ and 100 µg (Experiment I) or 50 µg (Experiment II) of electrophoretically purified pancreatic DNase. After 5 min of incubation at 37° the filters were washed with the same buffer, dried and counted.

DISCUSSION

Using the membrane filter technique we have demonstrated that the termination factor ρ binds to DNA molecules forming complexes which are retained on Millipore filters. From the binding curve (Fig. 2) it can be calculated that 1.6 µg of native E. coli DNA are bound by 0.5 µg of ρ. Assuming that all of the ρ factor is bound to the filters and the molecular weight of ρ is about 200,000 (2), a ratio of 1 molecule of ρ per

1000 base pairs is calculated. The binding of ρ to native DNA is a fast and reversible reaction, one native DNA molecule being replaced in the complex by another native DNA molecule (Table I and Fig. 3). In addition, ρ binds to denatured DNA, the binding being strong and less reversible, since a preformed complex of ρ with denatured DNA is hardly dissociated by native DNA (Fig. 3). A marked difference between the binding of ρ to native E. coli DNA as compared to native calf thymus DNA was observed. It may be speculated that a lesser number of binding sites exist for ρ on calf thymus DNA. This might be due to the presence of longer transcriptional units or to different termination signals with altered nucleotide sequences specific for a putative mammalian termination factor. If the latter hypothesis is correct, one can assume that the higher affinity of ρ to denatured DNA (Fig. 3) represents a non-specific interaction.

The effect of actinomycin D on the binding of ρ to DNA was studied in order to determine if guanine residues are involved in this interaction. As in the case of RNA polymerase-DNA binding (12, and our own unpublished observations), actinomycin D did not affect ρ-DNA complex formation. One may conclude that the binding sites for these two proteins are poor in guanine residues. It was shown (Table II) that ρ will confer partial protection to native E. coli DNA against DNase digestion. This observation coupled with the finding that the amount protected DNA reaches a plateau level above a certain concentration of ρ suggests the existence of discrete sites for the attachment of the termination factor. Assuming all of the ρ factor to be bound, it can be calculated that about 12 base pairs are protected per one molecule of ρ .

The results presented above support the hypothesis that ρ recognizes specific DNA sequences functioning as termination signals. By binding to these sites, ρ may stop the progress of the RNA polymerase molecules along the template and bring the transcription to a halt. This is in contrast to the action of σ which does not bind directly to DNA, but requires the presence of RNA polymerase in order to recognize the proper initiation sites. In addition to direct binding to DNA the ρ factor could recognize and interact with RNA polymerase as well (8). In this case a more complex interaction would be involved in the termination of the transcription process.

ACKNOWLEDGEMENT

This work was supported in part by U.S. Public Health Service Agreement No. 455144.

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