# PREFERENTIAL BINDING OF *E. COLI* RNA-POLYMERASE TO A–T RICH SEQUENCES OF BACTERIOPHAGE LAMBDA DNA

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Received 4 December 1970

## 1. Introduction

Upon binding to a DNA template, the bacterial RNA-polymerase promotes a) the retention of the DNA–RNA polymerase complex on membrane filters [1] and b) the protection towards nucleolytic digestion of a fraction of this DNA [2–6]. This fraction is assumed to represent the binding sites of the polymerase. The digested complex is itself retained on filter [3, 4, this work] which provides for a simple means of assaying the amount of DNA protected. Both the retention and the protection phenomena can be observed with or without the presence of one or several nucleoside triphosphates. The protection phenomenon is an obviously interesting potential tool for probing into the structure of those discrete sites on DNA at which transcription can be initiated. However, before a meaningful analysis of the promoter structure can be made, they must be unambiguously identified to the RNA-polymerase binding sites as obtained by the protection technique. Such evidence is still remote as it is well documented that there are much more binding sites for the polymerase than specific initiation sites [7]. This discrepancy can conceivably be reduced to some extent by using initiated complexes and increasing the ionic strength as only those complexes would not be dissociated by the salt [7–10]. However, initiation is not a satisfactory criterion of specificity. Indeed, the core polymerase lacking the sigma factor responsible for the specificity of transcription can still initiate and synthesize RNA though to a reduced extent [11].

We want to report here our attempts at limiting the number of non-specific binding sites and the base composition of the λ DNA protected regions obtained under these conditions. Our approach has been to work in a range of polymerase/DNA ratio corresponding to a very limited binding. We thus hope to restrict the binding to the sites of highest affinity for the polymerase [12] and which are more likely to be the physiological initiation sites *in vivo*.

## 2. Materials and methods

The polymerase was extracted from *E. coli* A 19 according to Babinet [13] and further purified by two cycles of glycerol gradient centrifugation [14]. The presence of sigma factor was checked by acrylamide gel electrophoresis [11]. Pancreatic DNase and venom phosphodiesterase were both obtained from Worthington. Pronase was from Calbiochem.

The labeled lambda phages were obtained by thermal induction of *E. coli* C 600 $C_1$ 857/λ grown in modified Kaiser's medium [16] containing 5% casaminoacids, $10^{-3}$ M $K_2H\,PO_4$. 20 mCi/l of $^{32}P$-$H_3PO_4$ (CEA, Saclay, France) were added at the time of induction. The phages were purified by phase partition [17], banded in cesium chloride and the DNA extracted by phenol at pH 7.5 in the presence of $4 \times 10^{-2}$ M EDTA. Acrylamide gel electrophoresis of protected DNA was performed according to Peacock and Dingman [15] using a 10% gel in the presence of 0.2% SDS.

For base composition analysis DNA was hydrolysed without carrier in $10^{-2}$ M tris HCl pH 8.0, $10^{-2}$ M $MgCl_2$, $10^{-3}$ M $CaCl_2$ for 1 hr at 37° by

10 μg/ml DNase I (electrophoretically purified, Worthington DPFF). After addition of glycine–NaOH buffer pH 9.4 to 0.05 M, NaF to $10^{-2}$ M and venom phosphodiesterase to 10 μg/ml the incubation was continued for another 30 min at 37°. The hydrolysate was diluted to 5 ml with water, 0.5 ml of a solution of cold nucleotides (200 μg/ml of dCMP and dAMP, 500 μg/ml of TMP and dGMP) added and the pH adjusted to 8.6 by $NH_4OH$. The four nucleotides were separated according to Attardi et al. [18] using scaled down Dowex 1 X 8 columns (0.4 X 3 cm). The eluted fractions were counted in Bray's solution.

## 3. Results

### 3.1. *Binding of polymerase and isolation of protected DNA*

Binding mixtures contained 4 X $10^{-2}$ M tris HCl pH 8.0, $10^{-2}$ M $MgCl_2$, $10^{-3}$ M $CaCl_2$ and varying amounts of $^{32}$P-DNA and polymerase. Incubation was at 37° for 5 min. Pancreatic DNase was then added to 100 μg/ml and incubated for 30 min at 37°. Venom phosphodiesterase was then added to the same concentration and incubation continued for an additional hour. The mixture was brought to 0.5% SDS, $10^{-2}$ M EDTA, treated by 100 μg/ml self digested pronase for 15 min at 37° and extracted three times with phenol saturated with 0.1 M tris HCl pH 9–$10^{-2}$ M EDTA. After addition of ¼ volume of a solution containing 3 mg/ml of each of the four deoxynucleosides triphosphates and NaCl to 0.1 M, the protected DNA was precipitated from the aqueous layer by two volumes of ethanol (overnight at –20°). The precipitate was spun down for 30 min at 20,000 *g*, dissolved in $10^{-2}$ M tris HCl pH 8, $10^{-3}$ M EDTA, 0.1 M NaCl, reprecipitated and finally dissolved in 0.5 ml $H_2O$. A translucent, readily soluble pellet is sometimes observed, probably containing some peptidic material resulting from the pronase digestion. Acrylamide gel electrophoresis of an aliquot showed a sharp peak with some smaller material. By comparison of its relative migration with that of a 4 S marker, a rough estimate of about 50 nucleotides was made for the length of the protected DNA in agreement with previous report [3].

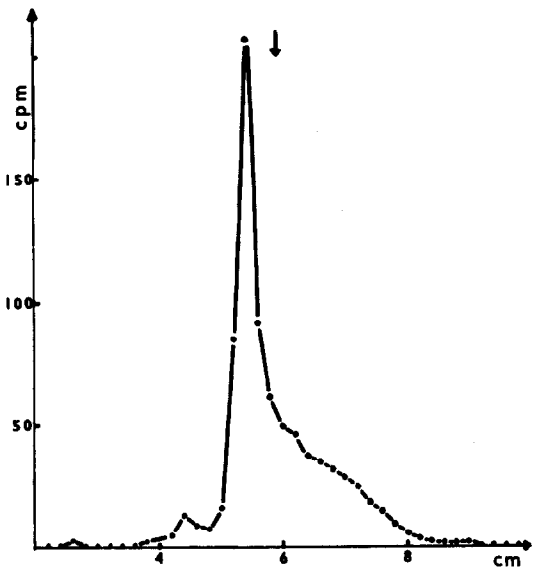


Fig. 1. Acrylamide gel electrophoresis of protected DNA. An aliquot of protected DNA obtained at low level of protection as described in Results 3–1 is adjusted to 0.2 ml $10^{-3}$ M tris HCl pH 8, $10^{-3}$ M EDTA and electrophoresed along with bromophenol blue on a 10% gel (0.7 X 12 cm) in 0.2% SDS under 10 mA. When the marker dye has reached the position indicated by an arrow, the gel is cut into 2 mm slices and the radioactivity determined using Cerenkov radiation.

### 3.2. *Base composition of protected DNA*

Table 1 shows the result of base composition analysis of protected pieces of DNA obtained at polymerase/DNA ratios corresponding to 3 different levels of protection. At low level of protection (0.1%), corresponding to a very limited number of polymerase molecules bound per genome (1 or 2 depending on the strandedness of the protected region), a significant enrichment in A–T as compared to total DNA is observed. When the amount of polymerase is increased, this difference is no longer observed. Similarly, the protected DNA obtained with a denatured template also reflects a random binding of the polymerase. Another feature of these protected DNA sequences is the rather good equality between A and T on one hand, G and C on the other hand. Although this observation cannot be taken as compelling evidence, it however suggests some degree of double strandedness for the binding sites to the native template.

Table 1
Base composition of protected λ DNA fragments.

| System | Number of experiments | % protection [a] | Binding sites per genome [b] | Base composition | | | | % A–T | % enrichment in A–T |
|---|---|---|---|---|---|---|---|---|---|
| | | | | A | T | G | C | | |
| Total λ DNA [c] (no polymerase) | 9 | 0 | 0 | 25[3] | 25 | 25 | 25 | 50 | 0 |
| Protected fragments obtained with native λ DNA template | 4 | 0.1 | 1–2 | 27.5 | 29.5 | 21.8 | 21.4 | 57.0 | 14 |
| | 1 | 11.8 | 118–236 | 26.1 | 25.1 | 25.1 | 23.7 | 51.2 | 2.4 |
| Protected fragments obtained with denatured λ DNA template | 1 | 11.4 | 114–228 | 26.5 | 25.3 | 24.5 | 23.6 | 51.8 | 3.6 |

[a] To determine the extent of protection an aliquot of the digested reaction mixture was filtered on Millipore (0.45 μm) washed with 40 ml $10^{-2}$ M tris HCl pH 8.0–$10^{-2}$ M $MgCl_2$ at 0° and assayed for the fraction of input radioactivity retained. Blanks obtained in the absence of polymerase (0.02–0.04%) were subtracted from all values.

[b] The number of binding sites per genome was derived from the level of protection taking $10^5$ nucleotides for the total lambda DNA molecule and 50 nucleotides for the length of the protected fragments. The twofold variation in the number of sites depends on the assumed strandedness of the protected segment. Double strandedness corresponds to the lowest value.

[c] To compare data obtained with different DNA preparations, whose apparent base composition may vary slightly, all values for total DNA have been normalized to 25% of each nucleotide and the values for the protected pieces corresponding to the same preparation corrected accordingly.

## 4. Discussion

We report here our attempts at limitimg the number of non-specific binding sites of RNA-polymerase to λ DNA and the base composition of the protected DNA fragments obtained under these conditions. Such an analysis had already been performed by Nakano and Sakaguchi [6] and failed to reveal any significant changes in the basic composition of the protected segments as compared to total DNA. However, their analysis was done on protected DNA pieces amounting 1.1% of total input DNA and corresponding to 9–18 sites per λ genome. This indicates that the binding sites they analysed must be mostly non-specific as there are only three to four early initiation sites [19,20]. At variance with their results, we find that RNA-polymerase does not bind randomly to λ DNA, provided limiting amount of enzyme is used. At this very low level of protection, we are likely to favor specific binding sites in two ways. First, at the time of the initial binding to the native DNA template, by selecting the few sites of highest affinity for the polymerase [12] and whose number (about 5) is rather close to that of known initiation sites. Second, by reducing the amount of free and reversibly completed polymerase molecules available for secondary binding to new sites (nicks or single stranded regions) artificially produced by the nuclease digestion.

A crucial point, as to the biological significance of our results, is to determine whether the sites we have analysed are the binding sites for the core of the complete enzyme. The enrichment in A–T we observe could conceivably reflect the increased dissociation of the sigma factor at this very low polymerase concentration. However, it has been shown that the stability of the polymerase–DNA complex depends on the binding temperature and on the presence of sigma [21]. As we have been using a sufficiently high incubation temperature (37°), the complexes we have analysed are likely to be sigma dependent. Unstable core enzyme complexes are expected to be strongly discriminated against by the nuclease treatment.

Our finding may have some relevance to the fact that poly dAT has a strong affinity for the RNA-polymerase [1] as well as for the lac repressor [22]. The preferential binding of both the polymerase and the repressor to A–T rich sequences might reflect

some degree of overlapping between the promoter and operator sites. This speculation draws some support from the fact that the repressor inhibits the binding of the polymerase to those promoters known to be under direct control of the repressor [23].

### Acknowledgements

We are indebted for his support to Dr. C. Paoletti in whose laboratory this work was acheived with the financial aid of the Ligue Nationale Française contre le Cancer and the Commissariat à l'Energie Atomique (C.E.A.) We are grateful to Mlle S. Colin for her expert technical assistance and to Mr. D. Toutain for his help in growing phage stocks.

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