BINDING SITES OF *E. COLI* RNA POLYMERASE ON T_7 DNA AS DETERMINED BY ELECTRON MICROSCOPY

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1. Introduction

The genetics of the regulation and expression of the early and late genes are best studied in *E. coli* bacteriophage T_7 [1]. In vivo and in vitro transcription of its DNA by *E. coli* RNA polymerase starts at the left end [2,3] where at least three different initiation sites have been detected [4–12]. Dunn & Studier [12] have calculated the positions of these initiation sites with respect to the known position [13] of gene 0.3 by determining the sizes of the small RNA pieces obtained after cleavage by RNase III. Attempts to localize the initiation sites by electron microscopy have been made using different techniques by three different groups [14–16]. In this paper we describe the positions of the strong binding sites of RNA polymerase on T_7 DNA with a measuring precision in the order of ± 20 base pairs, using a protein free DNA spreading method [17] for electron microscopy. Similar attempts have been made by Bordier & Dubochet (personal communication).

2. Materials and methods

Glutardialdehyde kept under nitrogen in an 8% solution was obtained from Polysciences, Warrington, R. I., USA. Ethidium bromide was obtained from Sigma. All other reagents were analytical grade chemicals.

E. coli RNA polymerase was purified as described earlier [18] except that the high and low salt zonal centrifugation was run in inversed order. On SDS polyacrylamide slab gel electrophoresis in a gradient gel stained with Commassie brilliant blue the enzyme showed the four bands and one additional band with a mol. wt. of about 100 000. This 'impurity' represents less than 5% of the total protein content. The σ content of the enzyme was about 70%. Protein concentration was determined as described elsewhere [19]. The integrity of the phenol extracted T_7 DNA was examined by neutral and alkaline succrose gradient centrifugation.

DNA and enzyme were incubated at 37°C for 15 min in 0.03 M triethanolamine–HCl, pH 7.9, 0.05 M KCl, 0.008 M magnesium acetate. The sample was then fixed for 10 min at the same temperature by adding one fifth of the volume of fixation buffer (0.5% glutardialdehyde in the above buffer, pH 7.9). The DNA–RNA polymerase complex was separated from unbound enzyme on Sepharose 4B kept at 37°C and eluted with 0.1% glutardialdehyde, 0.03 M triethanolamine–HCl, pH 7.9 and 0.008 M magnesium acetate. The DNA containing fraction was prepared and photographed in the electron microscope as described earlier [17]. Control experiments were done using buffer instead of RNA polymerase.

Length measurements DNA filaments magnified 100 000 × and mapping of RNA polymerase molecules bound to DNA were performed on a Hewlett Packard Digitizer, connected to a HP 9100 Calculator and a Plotter. The data were processed on a CDC and

a Siemens 4004 computer. For the orientation of the DNA molecules the procedure according to Davis and Hymann [14] was followed.

3. Results and discussion

Two sets of experiments were analysed, one with a molar enzyme (monomer) to DNA ratio of 3.5 and one with a ratio of 8.8. Since the positions of the RNA polymerase molecules were identical, the data of both sets were combined. Fig. 1 shows an example of a complex from the experiments using a molar ratio of 8.8. One can clearly recognize four RNA polymerase molecules close to one end of the DNA.

For the average length of 175 DNA molecules measured we obtained 13.25 ± 0.16 μm. For the mapping of the RNA polymerase binding sites 225 DNA fibres with 448 enzyme molecules bound to it were analyzed. Fig. 2 shows the histogram obtained over the whole length of the DNA molecule with a class size of 0.083 μm. The insert illustrates the distribution on the left end of the DNA molecule with a class size of 0.0083 μm. This later class size is justified by the fact that the last significant bit of the measurements represents 0.0027 μm.

Four distinct peaks can be recognized within the left 2% of the T_7 DNA molecule. When we analyzed the data by fitting four Gaussian distributions, we obtained the following positions: 0.0672 ± 0.0157 μm; 0.1555 ± 0.0139 μm; 0.2018 ± 0.0099 μm; 0.2422 ± 0.0047 μm. The area of the individual peaks (100% = enzyme molecules bound within the first 1 μm of the DNA filament) gave the following respective values: 27.81%; 27.57%; 19.85%; and 24.77%. The example in fig. 3 shows three specifically and one unspecifically bound RNA polymerase molecules.

Three of the located binding sites were already predicted by the results of Dunn & Studier [12] at 0.5.; 1.1; and 1.5% of the total length of the DNA. We find these positions at 0.57 ± 0.12%; 1.17 ±

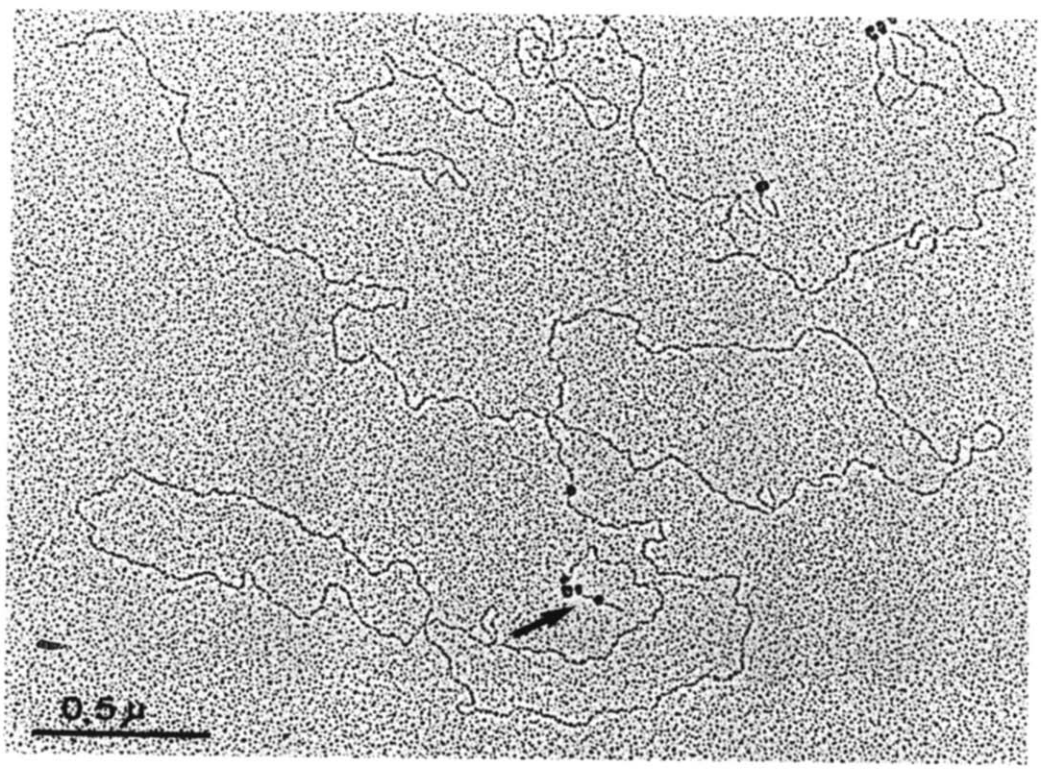

Fig. 1. Complex of RNA polymerase and T_7 DNA, formed with a molar enzyme per DNA ratio of 8.8. Note four RNA polymerase molecules bound close to one end of the DNA filament.

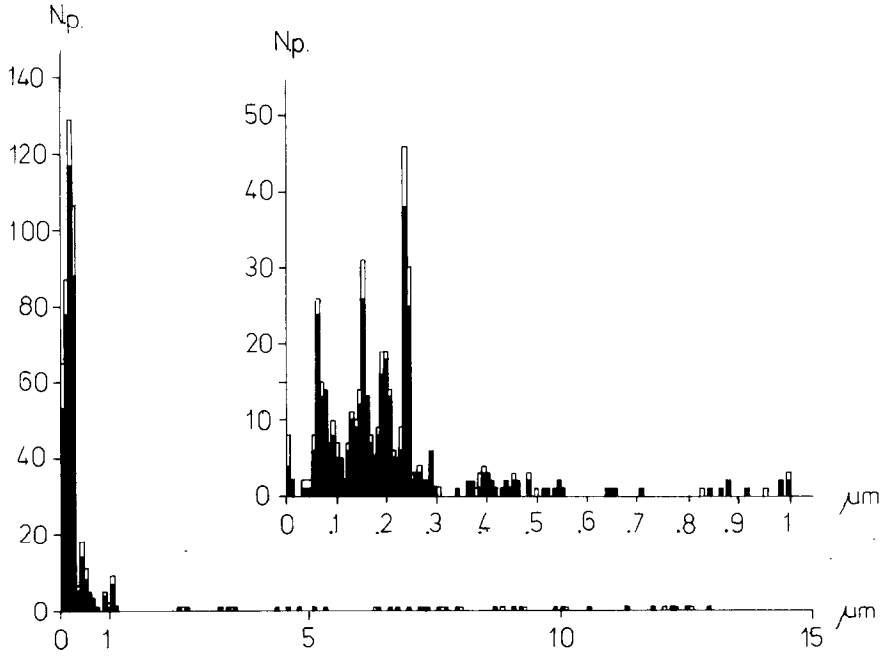

Fig. 2. Histogram of RNA polymerase molecules bound to T_7 DNA. Ordinate: Number of enzyme molecules. Abscissa: length in μm. 225 DNA filaments with 448 enzyme molecules bound to it were analyzed. Average length of DNA was 13.25 ± 0.16 μm (175 molecules). The peak on the 'left' side of the T_7 DNA molecule is shown in the insert with a class size of 0.0083 μm. The four peaks have the following positions: 0.0762 ± 0.0157 μm (= 0.57 ± 0.12% relative length with respect to 13.25 μm of the entire DNA molecule); 0.1555 ± 0.0139 μm (= 1.17 ± 0.10%); 0.2018 ± 0.0099 μm (= 1.52 ± 0.07%); 0.2422 ± 0.0047 μm (= 1.83 ± 0.04%). White boxes: Alternative sites of polymerase molecules in case of DNA crossover.

0.10% and 1.52 ± 0.07% relative length with respect to the whole DNA molecule (fig. 2). However, we observe an additional binding site at 1.83 ± 0.04%. It was shown that the first chain is starting with ATP, the second with GTP and the third again with ATP [10, 12]. Since the biochemical data are so clear, it seems that there is no place for an additional binding site. However, all the A to G ratios measured with [γ-^{32}P] nucleoside triphosphates in the literature [4–11] are reported as being larger than 2. Furthermore since our binding site at 1.83% is located very close to the gene 0.3 [13], one can argue that this short piece of RNA, which would result after cleavage with RNase III, could not be detected on a slab-gel as were the others. With the above arguments in mind, one could predict that an enzyme at this site starts with ATP.

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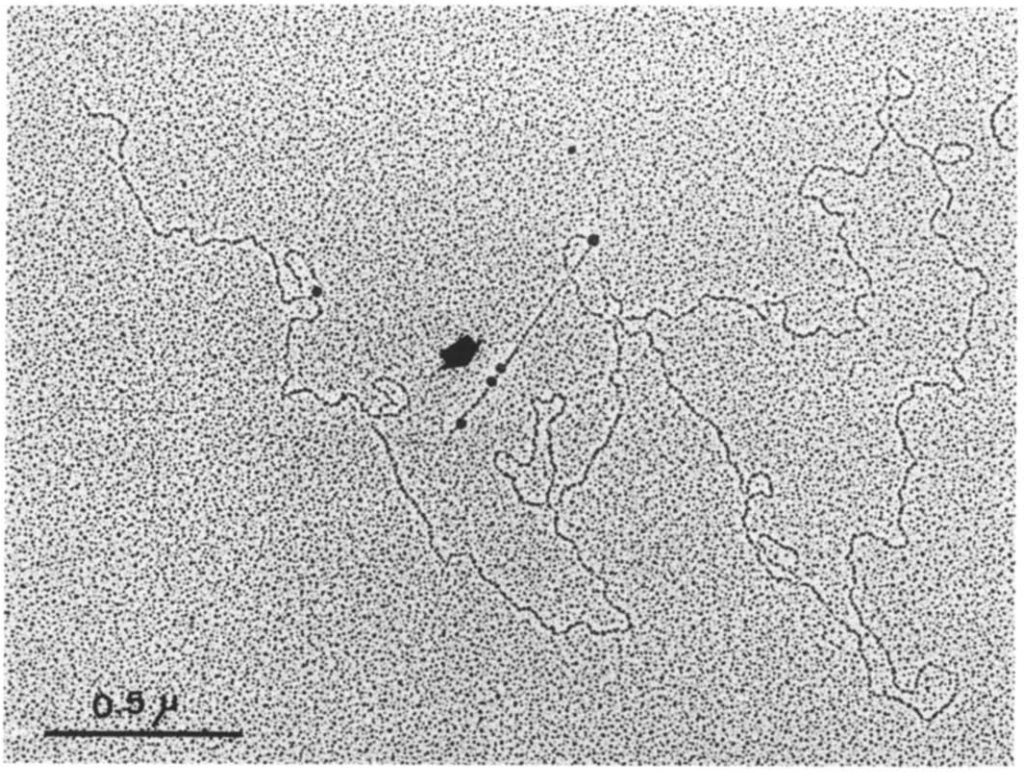

Fig. 3. Complex of RNA polymerase and T_7 DNA, formed with a molar enzyme per DNA ratio of 8.8. Three specifically and one unspecifically bound enzyme molecules are visible.

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