# SYNTHESIS OF RNA ON THE AT-RICH AND GC-RICH FRAGMENTS OBTAINED FROM BACTERIOPHAGE f1 DNA

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## 1. Introduction

Synthetic poly dAT has a strong affinity for *E. coli* RNA polymerase [1] and is a more active template for RNA synthesis than natural DNA and poly dG:dC [2–4].

We have isolated two sorts of hairpin-like structure, one rich in adenine–thymine pairing (AT-rich fragments) and another rich in guanine–cytosine pairing (GC-rich fragments), from bacteriophage f1 DNA [5, 6]. These fragments are almost equal molecular size (35–50 nucleotides).

In this communication, binding of *E. coli* RNA polymerase to the AT-rich and GC-rich fragments and synthesis of RNA on them was investigated. RNA polymerase of *E. coli* prepared by the method of Chamberlin and Berg was used as test material. The RNA polymerase bound more abundantly to the AT-rich fragments than to the GC-rich fragments, and RNA synthesis was more rapid and greater on the AT-rich fragments than on the GC-rich fragments. Furthermore, we wished to show the effect of phleomycin and actinomycin D on the RNA synthesis on both fragments.

## 2. Materials and methods

RNA polymerase was prepared from *E. coli* B cells by the modified method of Chamberlin and Berg [7] as described previously [8]. This preparation showed a sedimentation coefficient of 22 S and had a specific activity of 3 000 units/mg protein.

[$^{14}$C]Thymidine-labeled and cold AT-rich and GC-rich fragments were prepared by the methods reported previously [5, 6, 8]. The GC percentages of the AT-rich and GC-rich fragments were 38.7 and 58.7, respectively, and both preparations displayed reversible thermal denaturation.

Phleomycin (Lot no. NSC 61582 of Bristol Laboratories, Syracuse, N.Y.) was a gift from Dr. S. Suzuki of this institute and actinomycin D (Sigma Chem. Co.) from Dr. T. Kaneko of this laboratory. These antibiotics were dissolved in water and stored at −20°.

Experimental methods are described in the legends to the figures.

## 3. Results

### 3.1. *Binding of E. coli RNA polymerase to AT-rich and GC-rich fragments*

This subject was investigated by the sucrose density gradient method. Fig. 1 indicates that *E. coli* RNA polymerase is able to bind to both AT-rich and GC-rich fragments, but binding to the former is quantitatively greater. This difference in response was shown to be more marked when 7 μg RNA polymerase were reacted with 1 μg AT-rich or GC-rich fragments.

### 3.2. *Synthesis of RNA on AT-rich and GC-rich fragments*

Five μg *E. coli* RNA polymerase were reacted with 1 μg AT-rich or GC-rich fragments and the mixture was incubated at 37° for indicated periods. Amount of RNA synthesizing during the incubation

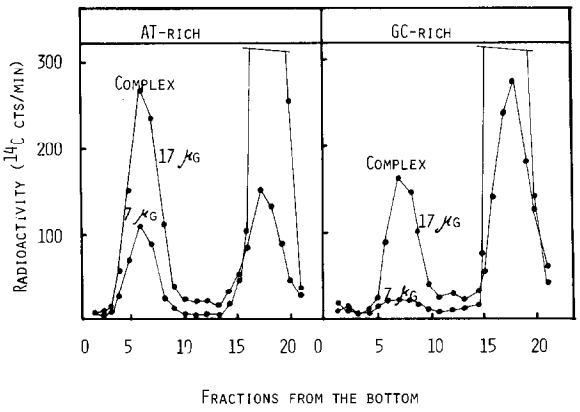


Fig. 1. Binding of RNA polymerase to AT-rich and GC-rich fragments. One μg (about 2 000 cpm) of radioactive AT-rich or GC-rich fragments was dissolved in 0.25 ml of binding buffer consisting of 10 μmoles of Tris-HCl (pH 8.0), 1 μmole of $MgCl_2$, 1 μmole of KCl, 3 μmoles of β-mercaptoethanol, and the indicated amount of RNA polymerase. The mixture was incubated at 37° for 5 min. After the reaction, the mixture was layered on a sucrose density gradient (3 to 15% sucrose dissolved in the buffer described above). The centrifugation was performed in a Hitachi model 55PA ultracentrifuge at 39,000 rpm for 15 hr at 4° using RPS 40 rotor. 0.23 ml fractions were collected from the bottom of the tube and the radioactivity was counted with a dioxane-based liquid scintillator in a Beckman DPM 100 type liquid scintillation spectrometer.

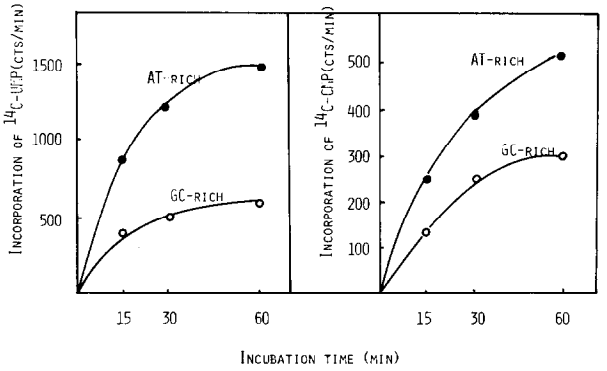


Fig. 2. Synthesis of RNA on AT-rich and GC-rich fragments. The reaction mixture contained, in 0.25 ml: 10 μmoles of Tris-HCl (pH 8.0), 1 μmole of $MgCl_2$, 1 μmole of KCl, 3 μmoles of β-mercaptoethanol, 250 mμmoles of $MnCl_2$, 100 mμmoles each of ATP, CTP, GTP and $^{14}$C-labeled UTP ($1.5 \times 10^6$ cpm/μmole) or 100 mμmoles each of ATP, GTP, UTP and $^{14}$C-labeled CTP ($1.0 \times 10^6$ cpm/μmole), 1 μg of cold AT-rich or GC-rich fragments, and 5 μg of RNA polymerase. Nucleoside triphosphates were purchased from Sigma Chem. Co. The $^{14}$C-labeled UTP and CTP were purchased from Schwartz BioResearch, USA. The mixture was incubated at 37° for indicated period and the reaction was terminated by addition of trichloroacetic acid.

was estimated from the amount of $^{14}$C-labeled UMP or CMP converted to a TCA-insoluble form. Fig. 2 shows that the synthesis occurs more rapidly and to a greater extent on the AT-rich than on the GC-rich fragments. Molar percentages of adenine in the AT-rich and GC-rich fragments were 29.3 and 19.1, respectively, and molar percentages of guanine were 19.8 and 31.5, respectively. The less enzyme is used in reaction, the more dominant as a template do the AT-rich fragments appear (not shown in figure).

### 3.3. *Synthesis of RNA in the presence of phleomycin or actinomycin D*

Phleomycin has been reported to bind specifically to the AT pairs in DNA [9–12], whereas actinomycin D is known to bind specifically to the guanine residues in the helical configuration of DNA [13–16]. An experiment similar to that in fig. 2 was conducted in the presence of various concentrations of phleomycin or actinomycin D. Fig. 3 shows that RNA synthesis on the AT-rich fragments is inhibited more effectively by phleomycin than on the GC-rich fragments and, on the contrary, RNA synthesis on the GC-rich fragments is inhibited to a greater extent by actinomycin D than on the AT-rich fragments. These results seem to reflect the differences of base composition and base sequence between two sorts of fragments. But, the degree of inhibition of RNA synthesis by phleomycin is lower than that by actinomycin D.

## 4. Discussion

Binding of RNA polymerase to a specific region on a DNA strand is believed to be the first step of RNA synthesis. The following experiments suggest that, as far as *E. coli* RNA polymerase is concerned, it may preferentially bind to a region rich in adenine–thymine pairing. i) The DNA segments protected from nuclease digestion by forming a complex between *E. coli* RNA polymerase and phage λDNA were rich in adenine and thymine residues [17]. ii) *E. coli* DNA polymerase formed complexes to a greater

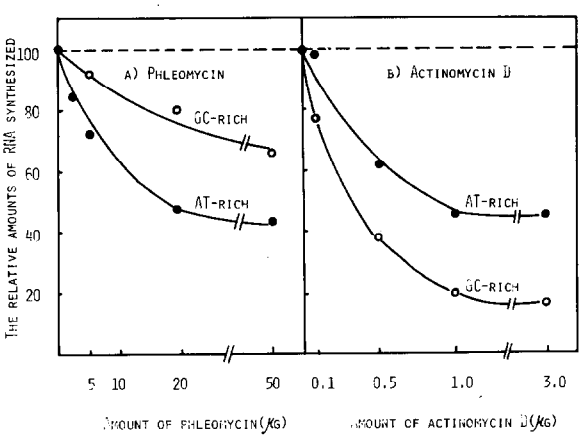


Fig. 3. Effects of phleomycin and actinomycin D on synthesis of RNA on AT-rich and GC-rich fragments. The RNA synthesis was investigated using the reaction mixture shown in fig. 2 in the presence or absence of antibiotic. The relative amount of RNA synthesized during the incubation at 37° for 60 min was estimated from the count of radioactivity which each mixture displayed. The total values in the absence of antibiotic were 1,634 cpm (AT-rich) and 560 cpm (GC-rich) in fig. 3a ([$^{14}$C]UMP) and 528 cpm (AT-rich) and 309 cpm (GC-rich) in fig. 3b ([$^{14}$C]CMP.

extent with synthetic poly dAT than with natural DNA and poly dG:dC [2–4]. iii) The binding of *E. coli* RNA polymerase to the replicative form of DNA bacteriophage f1 was inhibited markedly by the co-existance of AT-rich fragments and phleomycin [8]. The result shown in fig. 1 may be regarded as an additional support of this concept. The polymerase preparation bound more abundantly to the AT-rich fragments than to the GC-rich fragments.

Since the binding of RNA polymerase to DNA could not be followed by RNA synthesis, synthesis of RNA on the AT-rich and GC-rich fragments was investigated. Synthesis occurred more rapidly and to a greater extent on the AT-rich fragments than on the GC-rich fragments even when the synthesis was estimated from the amount of [$^{14}$C]CMP incorporated into the RNA fraction (fig. 2b). Thus, *E. coli* RNA has a preference for AT-rich fragments as template for both binding and RNA synthesis.

The binding of RNA polymerase to the AT-rich fragments was inhibited considerably by phleomycin as reported previously [8] and it has been shown in this paper that the synthesis of RNA on the AT-rich fragments was also inhibited by the same agent (fig. 3). But, the degree of inhibition by this agent on RNA synthesis is lower than on the binding of RNA polymerase. It seems that phleomycin, which shows a specific binding to the AT pairs in DNA, inhibits strongly the binding of polymerase to the AT pairs in the AT-rich fragments which may play an important role in binding, but once RNA synthesis has started, this agent has less effect on chain elongation than on binding. Similar effect with phleomycin has been reported using proflavine [18] and Falaschi and Kornberg [9] reported that phleomycin strongly inhibited DNA synthesis as compared with RNA synthesis.

The GC-rich fragments can also act as template for binding and RNA synthesis. But, the activities are considerably smaller than those with AT-rich fragments. Actinomycin D inhibits markedly RNA synthesis by binding to the guanine residues in both sorts of fragments, whereas this agent does not inhibit effectively the binding of RNA polymerase as described previously [8]. A similar effect of actinomycin D on the binding has been reported by Richardson [18] and by Hyman and Davidson [19].

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