DIFFERENTIAL TRANSCRIPTION OF fd RFI DNA BY *CAULOBACTER CRESCENTUS* AND *ESCHERICHIA COLI* RNA POLYMERASES

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1. Introduction

Different DNA-dependent RNA polymerases (EC 2.7.7.6) purified from *Escherichia coli* and other bacteria reveal different features of transcription with the same template DNA [1–3]. For example, it has been demonstrated that different RNA polymerases synthesize different amounts of RNA from the same DNA [3]. These differences could be at least in part explained in terms of the number of promoter sites to be recognized by these RNA polymerases as well as of the length of the RNA products. In fact, it has been shown that specific transcription of template DNA by RNA polymerase is achieved by recognizing specific promoter sites on the template [4].

The present experiments were designed to determine whether two RNA polymerases, one from *E. coli* and the other from *Caulobacter crescentus*, recognized different promoter sites on RFI form of coliphage fd DNA. For this purpose, the sizes of RNA products synthesized by the two enzymes were compared.

We show here that *C. crescentus* RNA polymerase synthesizes four G-start RNA species in vitro from fd RFI DNA, one of which is unique in size for this enzyme. The other three are the same size as those synthesized by *E. coli* enzyme. These results and those on A-start RNA products are discussed particularly in terms of specific recognition of promoter sites by RNA polymerase.

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2. Materials and methods

2.1. *Bacteria, phage and growth conditions*

C. crescentus CB13B1a was grown at 30°C in nutrient Pye medium [5]. *E. coli* A19 was purchased from Oriental Yeast Co. These strains were used for preparation of RNA polymerases. *E. coli* K38 was grown at 37°C in complete medium [6], and used for the host cell of phage fd.

2.2. *DNA*

fd RFI DNA was prepared as in [7]. The RFI DNA fractions were further purified by CsCl/ethidium bromide equilibrium centrifugation [8]. No detectable RFII and RFIII DNAs were found in the RFI DNA preparation on 0.8% agarose gel electrophoresis [9]. One A_{260} unit/ml of the RFI DNA was ~5×10^4 p.f.u./ml on lysozyme spheroplasts.

fd single stranded (ss) DNA was prepared from the purified fd particles by usual phenol extraction.

2.3. *RNA polymerases*

C. crescentus and *E. coli* RNA polymerases were purified as in [10] with slight modifications. The enzymes prepared had no detectable nuclease activities, and were ~95% pure on SDS–polyacrylamide gel electrophoresis [11]. The specific activity of *C. crescentus* and *E. coli* enzymes were 730 and 790 units/mg, respectively, on calf thymus DNA template: one unit of the enzyme activity is defined

as the amount of protein which catalyzes the incorporation of 1 nmol of the [^{3}H]UTP into RNA in 60 min at 37°C in the standard reaction mixture.

2.4. *[γ-^{32}P]ATP and [γ-^{32}P]GTP*

These, with spec. act. ~10 Ci/mmol, were prepared as in [12].

2.5. *RNA synthesis* in vitro

The standard reaction mixture for RNA synthesis in vitro contained 0.04 M Tris–HCl (pH 7.9), 0.05 M KCl, 8 mM $MgCl_2$, 0.1 mM β-mercaptoethanol. Each 0.3 ml of the reaction mixture contained 2.6 μg fd RFI DNA and 25 units of RNA polymerase (~32 μg, molar ratio of enzyme : DNA $\simeq$ 85:1). To detect even putatively weak promoter sites, we used excess enzyme. After adding 4 μM of [γ-^{32}P]ATP or [γ-^{32}P]-GTP and 40 μM of the other three ribonucleoside triphosphates to the reaction mixture, the initiation was allowed to proceed for 5 min at 37°C. Then 10 μg/ml of rifampicin (Sigma) and 200 μM each of four ribonucleoside triphosphates (in the case of table 1, UTP was replaced by [^{3}H]UTP (8000 cpm/nmol)) were added and the reaction mixture was incubated for another 30 min at 37°C. After the RNA synthesis, 10 μg/ml of pancreatic DNase I (Sigma) was added to the reaction mixture and it was incubated further for 10 min at 37°C. Then, 30 μg carrier yeast tRNA was added and the mixture was passed through Sephadex G-50 column (1 × 10 cm) and the RNA fraction eluted was collected and analyzed.

2.6. *Formamide gel electrophoresis*

To determine the chain lengths of the RNA species synthesized, they were subjected to electrophoresis [13] on 3.8% polyacrylamide–99% formamide gels (10 × 14 × 0.1 cm) together with RNAs of known size. The reference RNAs used were tobacco mosaic virus RNA (supplied by Ms M. Fukuda), ^{32}P-labeled *E. coli* 23 S and 16 S rRNAs and yeast tRNA, which are 6400, 3100, 1500 and 75–90 nucleotides long, respectively. After electrophoresis for 16 h at 50 V, the gels were stained, destained, dried and exposed to X-ray films (Kodak, X-Omat).

2.7. *RNA–DNA hybridization and elution of hybridized RNA*

After RNA synthesis in vitro and purification of the RNA products as above, half of each RNA solution was precipitated twice with 2.5 vol. ethanol at –20°C. The resulting precipitate was collected and subjected to electrophoresis (fig.2a,e). The other half of each RNA solution was hybridized to 10 μg fd ss DNA in 0.5 ml hybridization buffer [14] containing 0.5 M KCl and 10 mM Tris–HCl (pH 7.5). The hybridization mixture was passed through Millipore filter (type HA, 0.45 μm) and the filter was washed with 0.5 ml of the same buffer. The filtrate was precipitated as before and the precipitate was subjected to electrophoresis (fig.2b,f). Further, the filter was washed twice with 10 ml of the same buffer. To elute any hybridized RNA bound to filter, the filter was incubated in 0.5 ml elution medium (water:formamide, 30:70, v/v) [15]. Then the solution was similarly precipitated and the precipitate was subjected to electrophoresis (fig.2c,g).

3. Results

RNA synthesis in vitro was performed in the presence of rifampicin after initiation of transcription so that only one time initiation would occur. Table 1 shows the incorporation of [^{3}H]UTP, [γ-^{32}P]ATP, and [γ-^{32}P]GTP into trichloroacetic acid-insoluble materials by the *C. crescentus* and *E. coli* RNA polymerases on fd RFI DNA. The incorporation of [^{3}H]UTP, which measures the total RNA synthesized, shows a slightly lower transcriptional activity for the *C. crescentus* enzyme than for the *E. coli* enzyme. However, the incorporation of [γ-^{32}P]ATP and [γ-^{32}P]GTP, which measures

Table 1
Incorporation of [^{3}H]UTP, [γ-^{32}P]ATP and [γ-^{32}P]GTP by *C. crescentus* and *E. coli* RNA polymerases with fd RFI DNA under conditions of excess enzyme

RNA polymerase	Incorporation (pmol)		
	[^{3}H]UTP	[γ-^{32}P]ATP	[γ-^{32}P]GTP
C. crescentus	899	1.91	2.96
E. coli	1239	0.42	1.73

After RNA synthesis as in section 2, the reaction mixtures were treated with 10% trichloroacetic acid and passed through TM-2 filters (Toyo Roshi Co.). The filters were washed, and their radioactivity was assayed

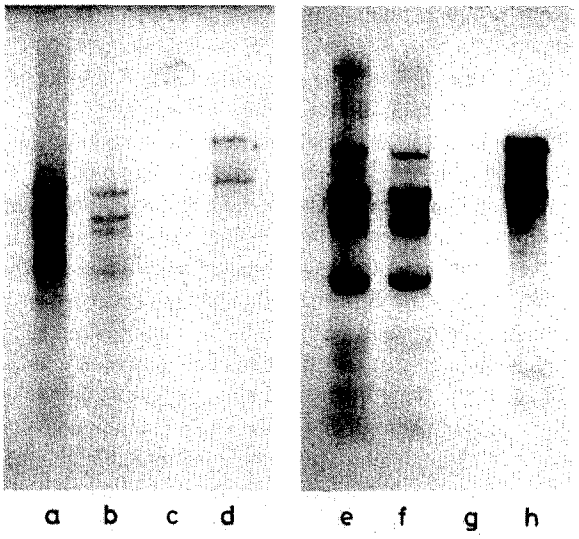

Fig.1. Autoradiograph of the RNA species synthesized in vitro by *C. crescentus* and *E. coli* RNA polymerases on fd RFI DNA. RNA synthesis was performed in the presence of [γ-^{32}P]ATP or [γ-^{32}P]GTP, and the RNA products were analyzed by formamide gel electrophoresis as in section 2. The arrows indicate the positions of migration of *E. coli* 23 S and 16 S rRNAs. The G-start RNA species synthesized by *C. crescentus* (a) and *E. coli* (b) RNA polymerases. The ^{32}P-labeled *E. coli* 23 S and 16 S rRNAs (c). The A-start RNA species synthesized by *C. crescentus* (d) and *E. coli* (e) RNA polymerases.

Table 2
Chain lengths of the G-start RNA species and relative strengths of their promoters

The G-start RNA species synthesized by *C. crescentus* RNA polymerase		*E. coli* RNA polymerase	
Chain length (nucleotides)	Relative[a] strength of promoter	Chain length (nucleotides)	Relative[a] strength of promoter
		2600	0.25
		2200	0.11
1400	0.89	1400	1.00[b]
		1100	0.18
920	1.08	920	0.53
730	0.64		
380	0.51	380	0.61

[a] The X-ray films were traced at $A_{600\ nm}$

The relative strength of the G promoters were then evaluated and expressed as a fraction of the strength of the [b]-marked promoter defined as the strength of 1.00

the number of RNA synthesized, by *C. crescentus* enzyme were 4.5- and 1.7-times higher than those by *E. coli* enzyme. This suggests that the *C. crescentus* enzyme can initiate transcription at more sites on the template than the *E. coli* enzyme.

To investigate the nature of this differential transcription by the two RNA polymerases, we analyzed the RNA products by formamide gel electrophoresis.

Figure 1 shows the electrophoretic patterns of A-start and G-start RNA species synthesized by the two enzymes. The *C. crescentus* enzyme synthesized at least four G-start RNA species, which were 1400, 920, 730 and 380 nucleotides long (fig.1a). The *E. coli* enzyme synthesized at least six G-start RNA species, which were 2600, 2200, 1400, 1100, 920 and 380 nucleotides long (fig.1b). Among the G-start RNA species, three have chain lengths common between the two enzymes and the others are specific for each enzyme. Furthermore, table 2 shows that the common

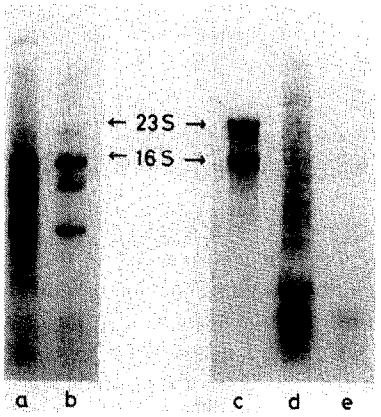

Fig.2. Autoradiograph of the G-start RNA species after hybridization to fd ss DNA. The experimental procedures were described in section 2. The G-start RNA species synthesized by *C. crescentus* RNA polymerase which were not submitted to the hybridization experiment (a), not hybridized (b) and hybridized (c) to fd ss DNA. The ^{32}P-labeled *E. coli* 23 S and 16 S rRNAs (d,h). The G-start RNA species synthesized by *E. coli* RNA polymerase which were not submitted to the hybridization experiment (e), not hybridized (f) and hybridized (g) to fd ss DNA.

RNA species were initiated at the strong promoter sites. Although there were discrete but weak bands, high background was found on the gel for A-start RNAs synthesized by the *C. crescentus* enzyme (fig.1d). This suggests that the incorporation of [γ-^{32}P]ATP by the enzyme is considerably random in initiation. The bands of A-start RNA species by the *E. coli* enzyme were also weak (fig.1e). Therefore, it was difficult to identify each A-start RNA species.

Figure 2 shows that all four G-start RNA species from the *C. crescentus* enzyme, like those from the *E. coli* enzyme, did not hybridize to fd ss DNA. (The discrete band in fig.2c was other RNA which could not be released from fd ss DNA even in the formamide system.) This indicates that all four, even the RNA species specific for the *C. crescentus* enzyme, were transcripts of the (−) strand of fd RFI DNA, like those by the *E. coli* enzyme [14]. A similar result was obtained for A-start RNA species. Therefore, recognition of promoter sites by the *C. crescentus* enzyme may be restricted to the (−) strand of fd RFI DNA.

4. Discussion

We have compared in vitro transcription of fd RFI DNA by *C. crescentus* RNA polymerase with that by *E. coli* RNA polymerase in terms of recognition of promoter sites. Transcription of the DNA and the related phage DNAs, f1 and M13 RFI DNAs, by the *E. coli* enzyme has been investigated [14,16–19]. The *E. coli* enzyme was shown to transcribe only the (−) strand of the template DNA. Further, they showed that the initiation of transcription occurs at several sites, while termination occurs at one unique site. However the number of promoter sites and their locations on the template are not in agreement among the investigators. Our results (fig.1b,e) were also only partially in agreement with them. The discrepancies may come from the differences in the assay systems used by different investigators.

We found that recognition of promoter and terminator sites by *C. crescentus* RNA polymerase is performed only on the (−) strand of fd RFI DNA like those by the *E. coli* enzyme. However, the RNA species synthesized by the two enzymes were shown to be different in numbers and some in sizes. Namely, the *C. crescentus* enzyme synthesized four G-start RNA species, while the *E. coli* enzyme synthesized six G-start RNA species. Among these RNA species, three had common chain lengths between the two enzymes and the others were specific for each enzyme. To synthesize the common RNA species, both enzymes must have recognized the same promoter and terminator sites on the template. To synthesize RNA species specific for each enzyme, however, each enzyme must have recognized different specific promoters, if the terminator site is unique for the template. Although the possibility of recognizing different termination sites by the two different enzymes cannot be neglected, the different transcriptional features observed between the two enzymes may well come from recognition of different promoter sites by the two enzymes.

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