Proc. Natl. Acad. Sci. USA
Vol. 78, No. 6, pp. 3454–3458, June 1981
Biochemistry

Changes in the promoter range of RNA polymerase resulting from bacteriophage T4-induced modification of core enzyme

(transcription control/role of σ subunit/*in vitro* transcripts/T4 tRNA genes)

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Communicated by E. Peter Geiduschek, March 3, 1981

ABSTRACT **Primary transcripts made *in vitro* on bacteriophage T4 DNA by RNA polymerase isolated from normal or T4-infected *Escherichia coli* were compared by gel electrophoresis. Bacteriophage-modified RNA polymerase fails to initiate transcription at certain promoters recognized by unmodified enzyme. In the T4 tRNA gene region, only one of the two promoters is active with the modified RNA polymerase. Reconstitution of separated RNA polymerase components demonstrates that this change in promoter site selection results from the modification of core enzyme and not σ factor.**

During the development of bacteriophage T4 in its host *Escherichia coli*, complex changes occur in the transcription process. These changes include the shutoff of host transcription and sequential expression of three classes of bacteriophage genes, served by "early," "middle," and "late" promoters. The host RNA polymerase (nucleosidetriphosphate:RNA nucleotidyltransferase, EC 2.7.7.6) is apparently used for all transcription throughout bacteriophage infection, and it is generally believed that at least some of the changes in the transcription pattern are achieved through phage-induced changes in RNA polymerase specificity (for review, see ref. 1).

Several modifications of RNA polymerase have been observed after T4 infection. They include chemical modification of the existing RNA polymerase subunits (2–13) and association with the enzyme of four small T4-coded polypeptides (14–20). Two of these new polypeptides (M_r 12,000 and 22,000) are the products of T4 genes 33 and 55, which are required for the transcription of the late genes (15, 16, 18). No relationship has been established between other T4-induced RNA polymerase modifications and switches in the transcription pattern.

Clearly, progress in understanding the molecular mechanisms of T4 transcription control depends on the development of *in vitro* systems that link particular RNA polymerase modifications with changes in transcription selectivity. Purified *E. coli* RNA polymerase *in vitro* recognizes only early promoters (21–23). *In vitro* transcription from middle (24) and late (25, 26) promoters has been demonstrated only in crude lysates of T4-infected cells. The study of functional changes in T4-modified RNA polymerase by using purified transcription systems has thus far produced limited information. The modified enzyme competes poorly with the host RNA polymerase for template DNA (27), has a higher transition temperature for rapidly initiating transcription complexes (28), and is inhibited by 0.2 M KCl (16). In contrast, the activity of the host RNA polymerase is markedly stimulated by KCl. The salt sensitivity of the modified enzyme is caused by a T4-coded polypeptide (M_r 10,000) that is associated with σ factor. RNA polymerase containing this protein fails to initiate transcription on T4 DNA at 0.2 M salt; this effect, however, can be overcome if 1% Triton X-405 is present at the moment of initiation (19, 20). It has been shown that modified RNA polymerase transcribes certain bacterial genes with reduced efficiency (29, 30), but no change in transcription selectivity was shown with respect to T4 genes.

A recently developed technique of electrophoretic separation of primary *in vitro* T4 transcripts (31, 32) allows direct analysis of promoter site selection by purified RNA polymerases. Two of the *in vitro* T4 transcripts made by host polymerase have been identified as products of the T4 tRNA gene cluster (see Fig. 1). They are initiated at different promoters, P_1 and P_2, and terminated at the same terminator site (33, 34). The experiments reported here show that T4-modified RNA polymerase fails to recognize several of the T4 promoters including the P_2 promoter of the tRNA genes.

MATERIALS AND METHODS

Chemicals. Ribonucleoside triphosphates and dinucleoside 3′–5′ monophosphates were from Sigma. [α-^{32}P]CTP was from Amersham. Triton X-405 was from Serva (Heidelberg).

Bacterial and Phage Strains. *E. coli* and bacteriophage T4 $psu_b^-\Delta 27$ mutant containing a deletion in the tRNA region (obtained from J. Abelson) were used. M9 medium/0.4% casamino acids was used for bacterial cultures (see ref. 14).

RNA Polymerases. Holoenzyme was purified from uninfected and T4-infected cells (10 plaque-forming units per cell, 18 min at 30°C) up to the ammonium sulfate step (modification II of the method described in ref. 27). This was followed by chromatography on DEAE-cellulose in TGED buffer (0.05 M Tris·HCl, pH 7.9/5% (vol/vol) glycerol/0.1 mM EDTA/0.1 mM dithiothreitol). Active RNA polymerase fractions eluted from the column by a 0.05–0.4 M KCl gradient were pooled, concentrated by precipitation with ammonium sulfate and further purified by gel filtration on Bio-Gel A-0.5 (Bio-Rad) in TGED buffer/0.5 M KCl. The enzyme preparations were analyzed by NaDodSO$_4$/polyacrylamide gel electrophoresis. The T4-modified polymerase contained 25–30% of the normal amount of σ factor. It also contained the four phage-coded polypeptides (see above). Both polymerase preparations contained several weak additional bands. However, the enzymes were not further purified to avoid further loss of σ from T4-modified enzyme.

Core enzymes and σ factors were separated by phosphocellulose chromatography as described (35). In the case of the host enzyme, the impurities were removed in the flow-through fraction, σ factor was recovered at the beginning of the KCl gradient, and core was eluted at ≈0.35 M KCl. In contrast, the σ factor from T4-modified polymerase was recovered together with the impurities in the flow-through fraction. As a result, the preparations of the host core, T4-modified core, and host σ factor were virtually pure, while σ factor from T4-modified polymerase was only 25–30% pure, as judged by NaDodSO$_4$ gel

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electrophoresis. The four phage-coded subunits were detected in the polymerase preparations from the infected cells, but their stoichiometry was not determined. All enzyme preparations were dialyzed against TGED buffer/50% (vol/vol) glycerol and stored at −20°C.

***In Vitro* Transcription System.** The standard 100-μl transcription system contained 10 μg of DNA/0.4 mM each ATP, GTP, UTP, and [^{32}P]CTP (0.02 Ci/mmol; 1 Ci = 3.7 × 10^{10} becquerels)/40 mM Tris·HCl, pH 7.9/10 mM $MgCl_2$/7 mM 2-mercaptoethanol. Two concentrations of KCl were used: 0.05 M (low salt) and 0.25 M (high salt) and, in some transcriptions, 1% Triton X-405 was added. The reaction was started by adding RNA polymerase to samples prewarmed to 37°C. After 3 min of incubation, rifampicin at 30 μg/ml was added and incubation was continued for 20 min. The reaction was stopped by adding actinomycin D at 50 μg/ml.

In the dinucleotide initiation experiment, RNA polymerase was incubated for 5 min at 37°C in the standard mixture without the four NTPs but with appropriate priming dinucleotide at 0.25 mM. ATP, UTP, GTP, and [^{32}P]CTP (2 Ci/mmol) were then added at 5 μM each. After 3 min, rifampicin at 30 μg/ml was added, and incubation was continued for 20 min. ATP, UTP, GTP, and CTP were then added to 0.4 mM, and the incubation was continued for another 10 min. The reaction was stopped as above.

The samples were treated with DNase I (RNase-free, Boehringer Mannheim), deproteinized with phenol, and prepared for electrophoresis as described (33).

Electrophoresis in 2.25% acrylamide/0.5% agarose gels was performed as described (33). The gel slabs were transferred to Siemens x-ray cassettes and autoradiographed at −70°C with Cronex (DuPont) intensifying screens.

RESULTS

In my experiments, DNA isolated from the bacteriophage T4 psu_b^-Δ27 deletion mutant was used as template for *in vitro* transcription. Deletion Δ27 (36) removes ≈1.3 kilobases of DNA from the internal part of the T4 tRNA gene cluster, resulting in the formation of two deletion-specific primary transcripts, band A_{27} RNA and band D_{27} RNA (1.6 and 2.1 kilobases, respectively) which represent two promoters in this genetic region (Fig. 1) and are easily identifiable because of their relatively small size. The *in vitro* transcription products were separated by gel electrophoresis and visualized by autoradiography.

In the experiment shown in Fig. 2, transcripts made by host (lanes 1, 3, and 5) and by T4-modified (lanes 2, 4, and 6) RNA polymerases under different conditions were compared. It can be seen that at 0.05 M KCl (lanes 1 and 2), the two enzymes synthesize the same set of RNA chains, including both transcripts of the tRNA cluster. At 0.25 M KCl (lanes 5 and 6), the activity of the host enzyme is markedly increased while the modified RNA polymerase is practically inactive. Lanes 3 and 4 show the transcripts made at 0.25 M KCl in the presence of 1% Triton X-405, which, in agreement with the results of Stevens and Rhoton (19, 20), relieves the salt inhibition of T4-modified RNA polymerase. It is clear that the host RNA polymerase synthesizes the same set of RNAs without (lane 5) and with (lane 3) Triton. There is a marked qualitative difference between the products of the two enzymes made in the presense of Triton at high salt (lanes 3 and 4); several bands, including band D_{27}, are not seen among the products of T4-modified polymerase, even at prolonged exposure times (data not shown).

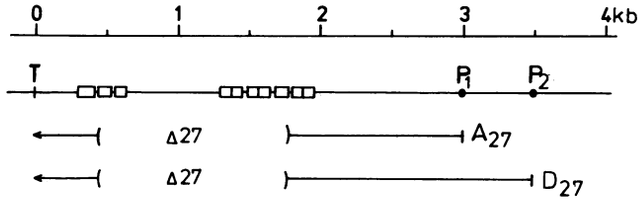

FIG. 1. Bacteriophage T4 tRNA gene cluster and its primary transcripts (33, 34). Open boxes represent genes of T4-specific stable RNAs that are arranged in two subclusters. The two transcription units in this region begin with promoters P_1 and P_2 and share a common terminator T. Parentheses indicate extent of deletion Δ27. Arrows represent the two transcripts—band A_{27} RNA and band D_{27} RNA—made by host RNA polymerase on T4 DNA containing deletion Δ27. The upper scale shows the distance (in kilobases) from the terminator.

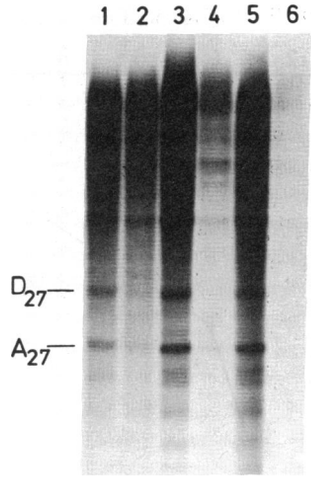

FIG. 2. Primary *in vitro* transcripts made by *E. coli* and T4-modified RNA polymerases. DNA (10 μg) was transcribed with 2.25 μg of host (lanes 1, 3, and 5) or 6.4 μg of T4-modified (lanes 2, 4, and 6) RNA polymerase. The reaction was carried out under standard conditions (see *Materials and Methods*) at 0.05 M KCl (lanes 1 and 2) or 0.25 M KCl (lanes 3–6) without (lanes 1, 2, 5, and 6) or with (lanes 3 and 4) 1% Triton X-405. Primary transcripts of the tRNA cluster, A_{27} and D_{27}, are indicated.

It is known that the relative amounts of transcription of different regions of T4 DNA depend to a considerable degree on the enzyme/template ratio; in particular, the RNA polymerase concentration needed for maximal transcription of the tRNA genes is one-fifth of that needed for transcription of other regions (37). Therefore, comparison of the two enzyme preparations should be made at the same concentration of active RNA polymerase molecules. This, however, was difficult to estimate because the T4-modified RNA polymerase preparation contained much less σ factor than the host enzyme (see *Materials and Methods*). Moreover, even at σ factor saturation, the modified polymerase has only one-third to one-half the specific activity of the host enzyme under the conditions used (data not shown). Therefore, the products made by the two RNA polymerases were compared at enzyme/template ratios ranging from large template excess to template saturation. Curves of DNA saturation with the two RNA polymerases, as judged from the incorporation of [^{32}P]CTP at high salt in the presence of Triton, are shown in Fig. 3. Under these conditions, RNA synthesis approaches a plateau at 12 μg of host enzyme or 30 μg of T4-modified polymerase per 10 μg of DNA. On the basis of these curves, five transcription samples for each enzyme were set up at various enzyme/template ratios (arrows in Fig. 3). The specific activity of [^{32}P]CTP in these samples was adjusted so that approximately the same amount of radioactivity would be incorporated in each sample, and the transcripts were analyzed by gel electrophoresis (Fig. 4). One can see that at all enzyme/

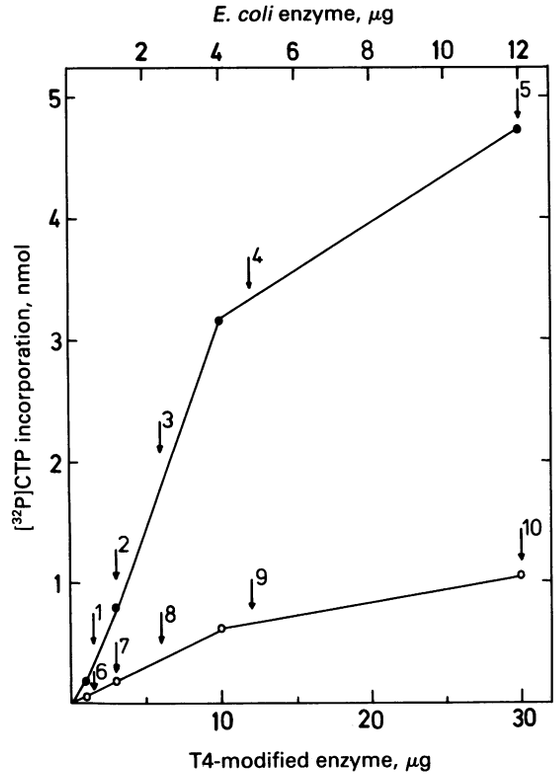

FIG. 3. Dependence of RNA synthesis on concentration of RNA polymerase in the sample. DNA (10 μg) was transcribed with different amounts of host (●) or T4-modified (○) RNA polymerase under standard conditions at 0.25 M KCl in the presence of 1% Triton X-405. Arrows indicate the points of the curves corresponding to the lanes in the gel slab (see Fig. 4).

template ratios tested, the T4-modified enzyme fails to synthesize certain species of RNA, including the D_{27} transcript of the tRNA cluster.

Different T4 transcripts made by the host polymerase can be selectively initiated with dinucleoside 3′–5′ monophosphate

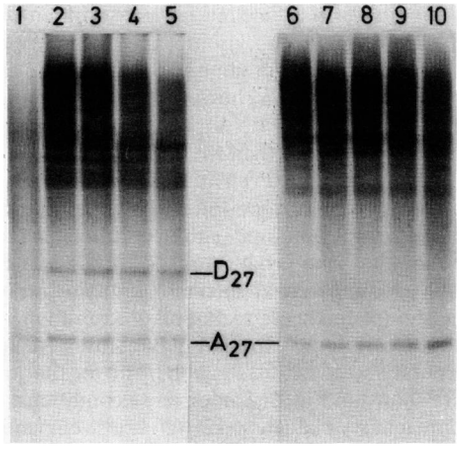

FIG. 4. *In vitro* transcripts made by *E. coli* (lanes 1–5) and T4-modified (lanes 6–10) RNA polymerases at different enzyme/template ratios. Transcription conditions were as described in the legend to Fig. 3. Specific activity of [^{32}P]CTP was adjusted so that approximately the same amount of label would be incorporated in each sample. The amount of enzyme in the samples is indicated by the arrows on the curves shown in Fig. 3.

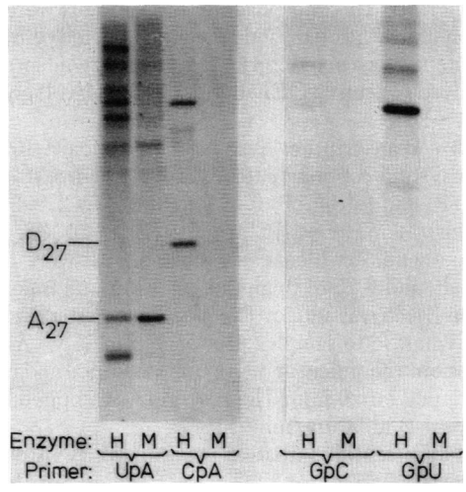

FIG. 5. Dinucleotide initiation of *in vitro* products of *E. coli* and T4-modified RNA polymerases. Host (H) (2.25 μg) enzyme or 6.4 μg of T4-modified (M) enzyme were used for transcription of 10 μg of DNA under dinucleotide chain initiation conditions (see *Materials and Methods*) at 0.25 M KCl in the presence of 1% Triton X-405.

primers (32, 34). Of the two promoters of the tRNA region, P_1 responds to initiation with UpA and P_2 responds to initiation with CpA (34). In the experiment shown in Fig. 5, host and T4-modified RNA polymerases were compared with respect to their ability to synthesize RNA chains initiated by different dinucleotides. One can see that the T4-modified polymerase fails to synthesize chains initiated by CpA, including band D_{27} RNA. In contrast, most of the transcripts initiated by UpA are synthesized by the modified enzyme, although some of them are produced in lesser amounts. However, more band A_{27} RNA is made by the modified polymerase than by the host enzyme under these conditions. The T4-modified enzyme practically does not synthesize RNA chains primed with GpU but makes one of the two weak transcripts primed with GpC.

The above experiments demonstrate that RNA polymerase from T4-infected cells does not synthesize certain RNAs made by unmodified host RNA polymerase. As both core enzyme and σ factor undergo modification after bacteriophage infection, it was interesting to determine which of the components of the modified polymerase is responsible for the changed range of enzyme products. For this purpose, the components of both host and modified polymerases were separated by phosphocellulose chromatography and then recombined. The incorporation of [^{32}P]CTP by the reconstructed enzymes under the conditions of core saturation with σ factor is summarized in Table 1. It is clear that the activity of σ factor from T4-modified polymerase depends on Triton at high salt to a much greater extent than that of the host σ, which is in agreement with the results of Stevens and Rhoton (19, 20).

The *in vitro* transcripts made by the reconstructed enzymes at high salt in the presence of Triton were compared by gel electrophoresis (Fig. 6). The results demonstrate that the inability to synthesize certain transcripts, including band D_{27} RNA, is associated with T4-modified core enzyme and not with σ factor.

DISCUSSION

As was observed by Stevens and Rhoton (19, 20), RNA polymerase isolated from T4-infected bacteria is not active at physiological ionic strength due to its inability to initiate transcrip-

Table 1. Reconstruction of RNA polymerase from separated core and σ components of host (H) and T4-modified (M) enzymes

Sample	Transcription conditions: Core	σ factor	Triton	[^{32}P]CTP incorporation, pmol
1	H	H	+	2360
2	H	M	+	2150
3	M	H	+	2050
4	M	M	+	1050
5	H	H	−	1120
6	H	M	−	400
7	M	H	−	840
8	M	M	−	120
9	H		+	80
10	M		+	40
11		H	+	10
12		M	+	40

Total RNA synthesis at 0.25 M KCl under standard conditions was determined with (+) or without (−) 1% Triton X-405. Host core (1.2 μg) and 4.0 μg of T4-modified core were used with saturating amounts of σ factors.

tion. This defect is caused by a small T4-coded polypeptide associated with the σ subunit of RNA polymerase. These experiments demonstrate that when the salt inhibition is relieved by 1% Triton, T4-modified polymerase fails to synthesize several species of RNA that are synthesized by unmodified host RNA polymerase. The absence of a particular RNA species from *in vitro* transcription products can, in principle, reflect an impaired chain termination. This, however, is not the case, at least with respect to the transcripts of the tRNA region whose terminator is efficiently utilized by T4-modified polymerase, as is evident from the formation of band A_{27} RNA. The failure of the modified enzyme to make D_{27} RNA, therefore, is likely to be due to its inability to utilize the P_2 promoter (see Fig. 1).

The enzyme-reconstruction experiment (Fig. 6 and Table 1) demonstrates that the change in the promoter range is associated with the core enzyme in the T4-modified RNA polymerase

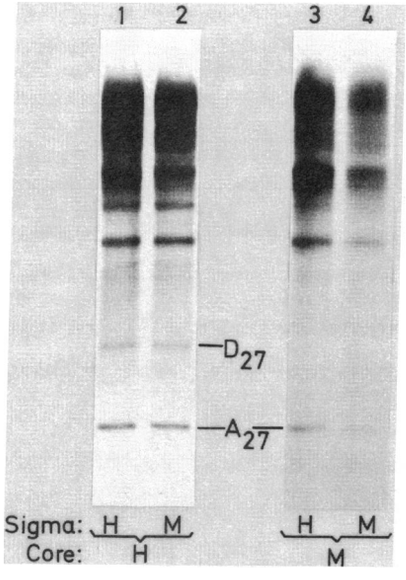

FIG. 6. *In vitro* transcripts made by enzymes reconstructed from host (H) and T4-modified (M) core and σ factor components. Numbers of the lanes correspond to samples 1–4 of Table 1.

and not with its σ factor. One can therefore conclude that although σ factor plays a critical role in promoter site selection (for review, see refs. 38 and 39), actual information for promoter recognition resides in the core enzyme. A similar conclusion was recently made by Davidson *et al.* (40), who compared promoter utilization on phage ϕ29 DNA by *E. coli* and *Bacillus subtilis* RNA polymerases.

No initiation from new promoters was observed with the modified RNA polymerase. This result is not surprising as these experiments were carried out with template DNA isolated from mature phage particles. However, it has been shown that, for transcription from middle (24) and late (1) promoters, modification of template DNA is required, along with changes in the transcription machinery.

The biological roles of the T4-induced change in RNA polymerase promoter range and of the salt-dependent inhibitor of initiation are not clear. Although the T4-modified RNA polymerase is not able to initiate transcription at high salt *in vitro*, its core at least is functioning normally *in vivo* despite the presence of inhibitory polypeptide in the σ factor. The change in the promoter range of the modified core may be connected with the shutoff of early gene expression at the late stage of bacteriophage development (41). This explanation implies that the promoters that are recognized by the modified core *in vitro* represent those early genes not shut off late in the phage infection. It is interesting that the genes of the tRNA cluster, which are actively transcribed at all times during phage development (42), have two promoters, one of which is not recognized by the modified core. This is consistent with the above model.

Alternatively, one can recall the hypothesis of Khesin *et al.* (27) about possible repressor function of RNA polymerase in shutting off early transcription. According to this hypothesis, RNA polymerase, which is unable to initiate transcription due to the presence of inactive σ factor, binds specifically to the promoters, making them unavailable for another active form of enzyme that transcribes middle and late genes. Consequently, the promoters that are not recognized by the modified core would not be subject to this type of repression. However, a preliminary attempt to reconstruct this model *in vitro* failed: the T4-modified polymerase preincubated with DNA at high salt without Triton had no effect on subsequent initiation of the standard set of RNA chains by the host of RNA polymerase. Therefore, the repressor model for the role of σ factor inactivation seems unlikely.

I am grateful to Prof. W. Zillig for his support and interest, to Alfons Gierl for analyzing my enzymes in a NaDodSO$_4$ gel, and to Siobhan Yeats for her comments on the manuscript. I am a recipient of a Max-Planck Fellowship.

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