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# Effects of GTP and ATP on the Transcription of T7 RNA Polymerase in the Linearized and Supercoiled DNA Templates

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**ABSTRACT:** The effects of GTP and ATP on the transcription termination and transcript production of bacteriophage T7 RNA polymerase were analyzed at the *thr* attenuator in the supercoiled and linearized DNA templates. T7 RNA polymerase and the supercoiled or linearized DNA template with the *thr* attenuator were preincubated with GTP or ATP first, then transcription was started by the addition of all four nucleotides. The results indicated that the termination efficiency of T7 RNA polymerase at the *thr* attenuator is significantly higher in the supercoiled DNA template than in the linearized one. With the preincubation of GTP, T7 RNA polymerase generates 4-21 times more RNA transcript in the supercoiled than linearized DNA template. Also, in the supercoiled DNA, T7 transcription with the preincubation of GTP produces 3.6-5.0 times more RNA transcript than that without preincubation. This strongly suggests that the topology of DNA template influences the transcript production of T7 RNA polymerase. Moreover, GTP may change the conformation of T7 RNA polymerase in the complex of transcription initiation.

**KEY WORDS: T7 RNA polymerase, Transcription termination, Supercoiled DNA.**

## INTRODUCTION

The RNA polymerase encoded by bacteriophage T7 is a monomeric protein, and can perform transcription initiation, elongation, and termination by itself (Chamberlin *et al.*, 1970). Because its simple system transcription carried out by T7 RNA polymerase is the best model to study the interaction among nucleic acids, T7 RNA polymerase, and other factors during transcription. Especially, the gene encoding T7 RNA polymerases has been cloned and overexpressed, and high quality T7 RNA polymerase is commercially available (Claire *et al.*, 1986; Davanloo *et al.*, 1984). It is easy to use T7 RNA polymerase to study its enzymatic activity *in vitro*. Consequently, T7 RNA polymerase can produce full length transcripts from DNA templates containing a T7 promoter, and vectors for the high level expression of genes cloned behind T7 promoter have been developed (Mead *et al.*, 1986) and widely used (Tanaka *et al.*, 1998; Sakakibara *et al.*, 1997).

T7 RNA polymerase has been reported to terminate its transcription, when it encounters the DNA sequence with the dyad symmetrical structure (Jeng *et al.*, 1990). The *thr* attenuator is structurally similar to other rho-independent terminator, and containing a G+C-rich dyad symmetry followed by a run of polydeoxythymidine (Gardner, 1982). It has been intensively studied with *E. coli* (Lynn *et al.*, 1985, 1987, 1988; Burton, 1989; Gardner, 1982; Yang and Gardner, 1989) and T7 (Jeng *et al.*, 1990, 1992, 1995, 1998) RNA polymerases. The effects of the concentrations of salts, magnesium chloride, and nucleoside triphosphates on the transcription of T7 RNA polymerases were analyzed (Jeng, 1995). The results

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indicated that transcription termination by T7 RNA polymerases was enhanced in the low concentrations of magnesium chloride or high amount of sodium chloride or potassium glutamate. Also, in response to low concentration of nucleoside triphosphates the termination efficiency of T7 was increased (Jeng, 1995).

The effects of the concentrations of nucleoside triphosphates on the transcription of bacteriophage T7 polymerases were also analyzed at the *thr* attenuator in the supercoiled and linearized DNA templates (Jeng and Lee, 1998). T7 RNA polymerase showed high termination efficiency in the low concentration of nucleoside triphosphates at the *thr* attenuator in the both linearized and supercoiled template. The *thr* attenuator in the supercoiled DNA template always caused more T7 RNA polymerase to cease transcription than that in the linearized DNA template. Also, when the concentration of ATP is limited from 5 $\mu$M to 150 $\mu$M within transcription reaction, the transcript production by T7 RNA polymerase was two folds more than that in the limited supply of any other nucleoside triphosphates. This result indicates that ATP may compete with GTP in affecting transcription of T7 RNA polymerase.

Previous study in our laboratory indicated that the concentration of nucleoside triphosphate affects T7 RNA polymerase on its transcription elongation and termination (Jeng and Lee, 1998). However, transcription initiation is the first step of transcription, and its efficiency is crucial for gene regulation. Therefore, the effects of nucleoside triphosphates on the T7 RNA polymerase in its promoter recognition are studied in this report. Also, the DNA within the cells is present mostly in supercoiled form. In this study, using *thr* attenuator as a model, the effects of the concentration of purine, ATP and GTP, on the transcription of T7 RNA polymerase are studied and compared within the linearized and supercoiled DNA templates.

## MATERIALS AND METHODS

### Materials

T7 RNA polymerase and *Eco*RI endonuclease were purchased from Bethesda Research Laboratories. [$\alpha$-$^{32}$P]CTP were purchased from Amersham Corp. at 410 Ci/mmol. Unlabeled ribonucleoside triphosphates were obtained from Sigma. Plasmid pTZ19thr with T7 promoter and the *thr* attenuator in the downstream was constructed as described (Jeng *et al.*, 1990), and prepared by ultracentrifugation in CsCl density gradient.

### *In vitro* transcription

Reactions (10 $\mu$L ) contained 10 pmole of supercoiled or *Eco*RI-linearized plasmid pTZ19thr, 10 units of T7 RNA polymerase, 1X reaction buffer (40 mM Tris-HCl (pH 8.0), 8 mM $MgCl_2$, 25 mM NaCl, 5 mM dithiothreitol), and 2-6 $\mu$Ci of [a-$^{32}$P]CTP (final specific activity = 0.13-0.4 mCi/nmol). If it was mentioned in each reaction 1$\mu$M GTP or ATP was added. Mixtures were incubated at 37℃ for 5 min before the four unlabeled ribonucleoside triphosphates, whose concentration was indicated in each reaction, were added to initiate the reactions. After 30 min at 37℃, the reactions were resuspended in 10 $\mu$l of 95% formamide, 0.1 % (w/v) xylene cyanol, 0.1 % (w/v) bromophenol blue, 50 mM Tris-HCl, 44 mM $H_3BO_3$ and 2.5 mM $Na_2EDTA$, and analyzed on 6% polyacrylamide with 8 M urea gels containing TBE buffer (50 mM Tris-HCl, 44 mM $H_3BO_3$, 2.5 mM $Na_2EDTA$) as described by Maxam and Gilbert (1980).

**Quantification of termination efficiency**

After electrophoresis gel was detected by the Phosphoimager from Molecular Dynamics, and the quantification of RNA fragments was performed by Imagequant software. The data were corrected for background and normalized according to the length and cytidine monophosphate (CMP) composition of the transcript. The termination efficiency of *thr* attenuator in the linearized pTZ19thr was calculated as (radioactivity in the threonine-terminated transcript) X 100/ (radioactivity in the threonine-terminated transcript + read-through transcript), and the total amount of transcript was the sum of radioactivity in the threonine-terminated and read-through transcripts (Jeng *et al.*, 1990).

The termination efficiency of *thr* attenuator in the supercoiled pTZ19thr was calculated as the following (Jeng and Lee, 1998). The radioactivity of the terminated transcript produced by T7 RNA polymerase at the *thr* terminator in the first time is assigned as THR. The total amount of RNA molecule produced by T7 RNA polymerase is indicated as T. The termination efficiency of T7 RNA polymerase at the *thr* attenuator is indicated as a%. C is the CMP composition of the terminated transcript, and B is the CMP composition of the read-through transcript. RT is the radioactivity in the read-through transcript, while R is the constant for the radioactivity used in reaction. When T7 RNA polymerase terminates at the *thr* attenuator in the first time, the radioactivity of this transcript, THR, can be estimated as T x a% x C x R and obtained from the terminated band in gel (Figs. 1 and 4). When T7 RNA polymerase goes around the whole sequence of plasmid pTZ19thr and terminates at the *thr* attenuator for the next following times, the radioactivity of these transcript, RT, can be obtained from the read-through band near the well of gel. Therefore, the radioactivity of the read-through band is calculated as $T \times a\% \times B \times R \times [(1\text{-}a\%) + 2 \times (1\text{-}a\%)^2 + 3 \times (1\text{-}a\%)^3 + 4 \times (1\text{-}a\%)^4 + \ldots]$, and equals to $T \times B \times R \times (1\text{-}a\%)/a\%$, that is RT. Since the values of C, THR, B, and RT can be obtained from experiments, a% and T can be calculated based on the equations $T \times C \times R \times a\% = THR$ and $T \times B \times R \times (1\text{-}a\%)/ a\% = RT$.

## RESULTS

The supercoiled pTZ19thr was obtained by CsCl-ethidium bromide isopycnic ultracentrifugation, and was estimated to contain over 95% pure supercoiled DNA from the analysis of agarose gel (data not shown). The linearized pTZ19thr was isolated from the *Eco*RI digestion of its supercoiled form, and the identity of its linearized was confirmed by 1% agarose gel (data not shown).

Since pTZ19thr contains a *thr* attenuator (Jeng *et al.*, 1990), the transcription termination occurs when T7 RNA polymerase reads through this region (Jeng *et al.*, 1990, 1992). For the *Eco*RI-linearized pTZ19thr, two transcripts were detected on the 6% urea-polyacrylamide gel. One is the 123 bp transcript terminated at the *thr* attenuator by T7 RNA polymerase, and the other is the read-through transcript produced by the run-off transcription of T7 RNA polymerase (Jeng and Lee, 1998). For the supercoiled DNA template, T7 RNA polymerase produces a 123 bp terminated transcript when it reaches the *thr* attenuator at the first time. Further, producing a group of high molecular weight transcripts (Figs. 1 and 4), when T7 RNA polymerase goes around the whole sequence of pTZ19thr and terminates at the *thr* attenuator for the next following times. Based on the equations listed in the Materials and Methods, both the termination efficiency and the total RNA transcript produced by T7 RNA polymerase can be estimated.

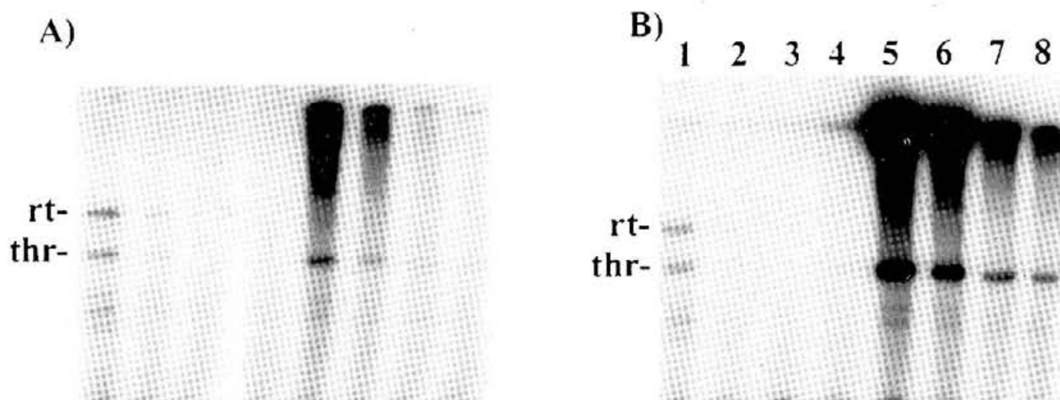


Fig. 1. Autoradiograph of the RNA transcription products in the presence of the different amount of GTP to initiate transcription. T7 RNA polymerase and the supercoiled or linearized pTZ19thr DNA template were incubated with or without 1 μM GTP at 37℃ for 5 min before the four unlabeled ribonucleoside triphosphates were added to initiate the reactions. The concentrations of these unlabeled nucleoside triphosphates were 1 mM for the CTP, ATP, and UTP, and the various amount of GTP indicated in each reaction. The terminated transcript (thr) and the read-through transcript (rt) are indicated. Panel A indicates the T7 transcription performed without the pre-incubation of GTP, and panel B is the T7 transcription preincubated with 1 μM GTP. Lane 1, 150 μM GTP in the linearized DNA; lane 2, 40 μM GTP in the linearized DNA; lane 3, 10 μM GTP in the linearized DNA; lane 4, 5 μM GTP in the linearized DNA; lane 5, 150 μM GTP in the supercoiled DNA; lane 6, 40 μM GTP in the supercoiled DNA; lane 7, 10 μM GTP in the supercoiled DNA; lane 8, 5 μM GTP in the supercoiled DNA.

**Effects of the GTP concentration on the transcription of T7 RNA polymerase**

The effects of the single nucleoside triphosphate on the transcription termination of T7 RNA polymerase have been studied (Jeng and Lee, 1998). However, whether the single nucleotide affecting the transcription initiation complex of T7 RNA polymerase has not been fully understood. Since the first three nucleotides of RNA transcript produced by T7 RNA polymerase on the pTZ19thr template is GMP, the effect of GTP on the transcription initiation of T7 RNA polymerase is studied here. T7 RNA polymerase and DNA template were incubated in the absence or presence of 1 μM GTP for 5 minutes, then all four ribonucleotides, whose concentrations were 1 mM for ATP, CTP, and UTP, and 150, 40, 10, or 5 μM for GTP, were added to start transcription (Fig. 1). The termination efficiency of T7 RNA polymerase at the *thr* attenuator is slightly higher in the supercoiled than linearized DNA template (Fig. 2). T7 RNA polymerase shows 50-64% termination efficiency in the linearized pTZ19thr DNA template, and 66-82% in the supercoiled form with or without the incubation of GTP (Fig. 2). This may indicated that the topology of DNA template affects the termination of T7 RNA polymerase at the *thr* attenuator.

The total amount of RNA transcript produced by T7 RNA polymerase was also measured (Fig. 3). Compared to in the linearized DNA template, T7 RNA polymerase produces more transcript in the supercoiled DNA template (Fig. 3). Especially, T7 RNA polymerase produces the most amount of RNA transcript in the supercoiled DNA template preincubated with GTP (Fig. 3). With the preincubation of GTP, T7 RNA polymerase produces 4-21 times more transcript in the supercoiled than linearized DNA template. This vividly suggested that the topology of DNA template influences the transcript production of T7 RNA polymerase. Even in the supercoiled DNA template, T7 RNA polymerase with the preincubation of GTP produces 3.6-5.0 times more RNA transcript than that without pre-incubation. However, the similar amount of RNA transcript was obtained in the linearized

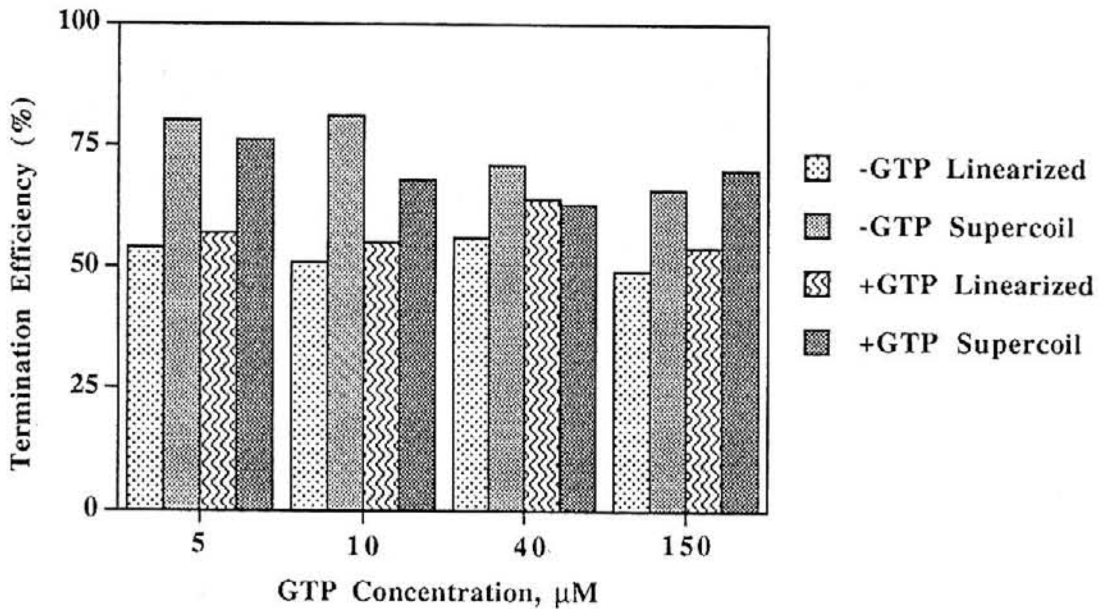


Fig. 2. Termination efficiencies of T7 RNA polymerase initiated by the different amounts of GTP. The transcription condition is described as in the legend of figure 1, and the termination efficiencies were determined as described in the text.

DNA template with or without the preincubation of GTP. This result may indicate that the preincubation of GTP with T7 RNA polymerase and the supercoiled DNA template, but not the linearized tempalte, greatly enhanced the production of RNA transcript by T7 RNA polymerase.

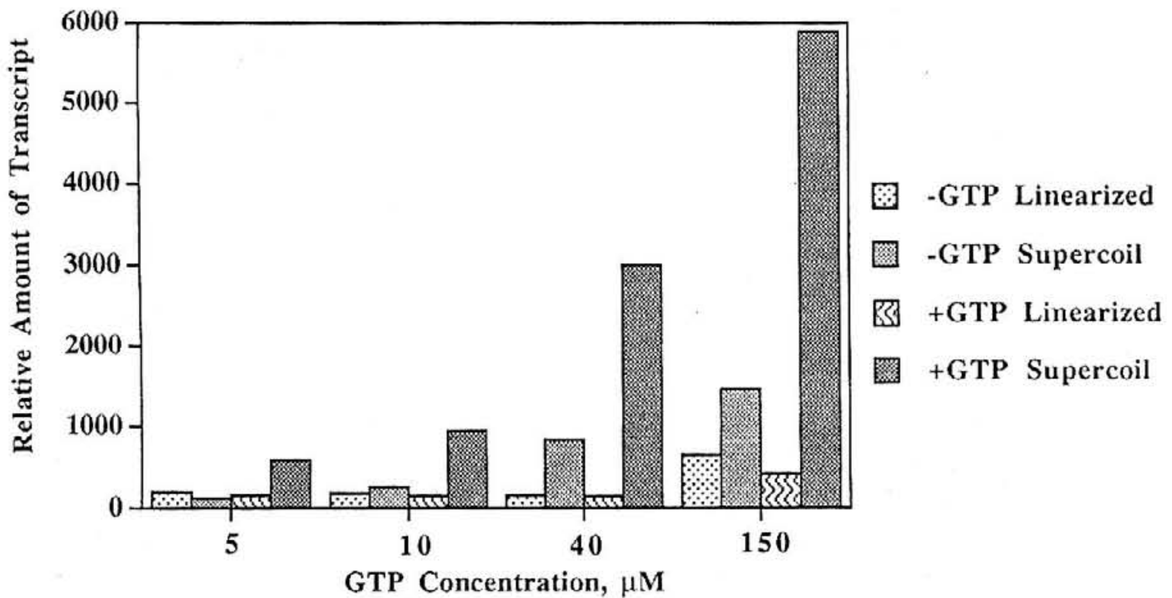


Fig. 3. Transcript production of T7 RNA polymerase initiated by the different amounts of GTP. The transcription condition is described as in the legend of figure 1, and the amount of transcript were determined as described in the text.

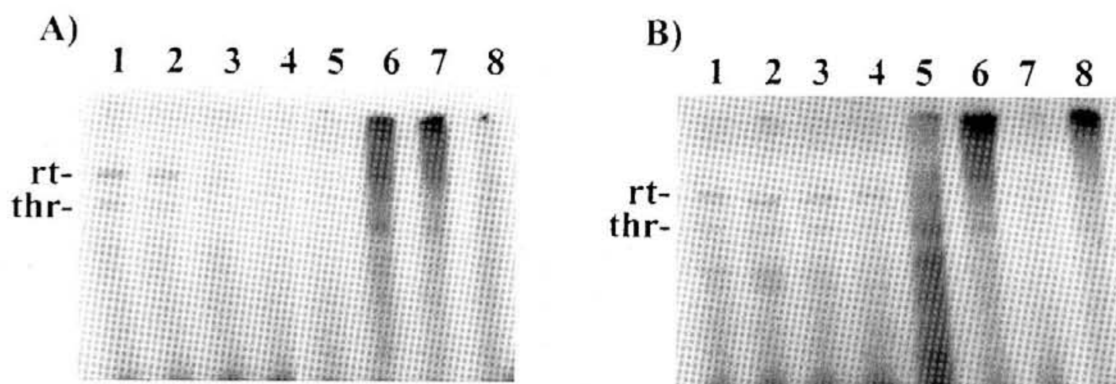


Fig. 4. Autoradiograph of the RNA transcription products in the presence of the different amount of ATP to initiate transcription. T7 RNA polymerase and the supercoiled or linearized pTZ19thr DNA template were incubated with or without 1 $\mu$M ATP at 37℃ for 5 min before the four unlabeled ribonucleoside triphosphates were added to initiate the reactions. The concentrations of these unlabeled nucleoside triphosphates were 1 mM for the CTP, GTP, and UTP, and the various amount of ATP indicated in each reaction. The terminated transcript (thr) and the read-through transcript (rt) are indicated. Panel A indicates the T7 transcription performed without the pre-incubation of ATP, and panel B is the T7 transcription preincubated with 1 $\mu$M ATP. Lane 1, 150 $\mu$M ATP in the linearized DNA; lane 2, 40 $\mu$M ATP in the linearized DNA; lane 3, 10 $\mu$M ATP in the linearized DNA; lane 4, 5 $\mu$M ATP in the linearized DNA; lane 5, 150 $\mu$M ATP in the supercoiled DNA; lane 6, 40 $\mu$M ATP in the supercoiled DNA; lane 7, 10 $\mu$M ATP in the supercoiled DNA; lane 8, 5 $\mu$M ATP in the supercoiled DNA.

### Effects of the ATP concentration on the transcription of T7 RNA polymerase

Since both GTP and ATP are purine, it is interesting to understand that if ATP shows the same effect as GTP does on the transcription of T7 RNA polymerase. The same reactions as described above were performed, except GTP was replaced by ATP in each reaction (Fig. 4). T7 RNA polymerase showed 48-58% termination efficiency in the linearized pTZ19thr DNA template, whereas 81-89% in the supercoiled DNA with or without the incubation of GTP (Fig. 5). The termination efficiency of T7 RNA polymerase at the *thr* attenuator is much higher in the supercoiled than linearized DNA template with the preincubation of ATP (Fig. 5). Again, topology of DNA influences the transcription termination of T7 RNA polymerase, and the higher transcription termination was always observed in the supercoiled than linearized DNA template.

When T7 transcription was initiated by 5 $\mu$M ATP, the same amount of transcript was produced in both supercoiled and linearized DNA templates with or without the pre-incubation of ATP. However, when the T7 transcription was started by the ATP, whose concentration equals to or is larger than 10 $\mu$M, some of the reactions with the supercoiled DNA template produced more RNA transcript than those with the linearized DNA template (Fig. 6). This may indicate that unlike the preincubation of GTP, T7 RNA polymerase preincubated with ATP was slightly affected by the topology of DNA.

## DISCUSSION

Transcription initiation includes promoter recognition by RNA polymerase and the polymeration of the first few nucleotides. The interactions among T7 promoter, T7 RNA polymerase, and nucleoside triphosphates have been footprinted by DNase I (Ikeda and

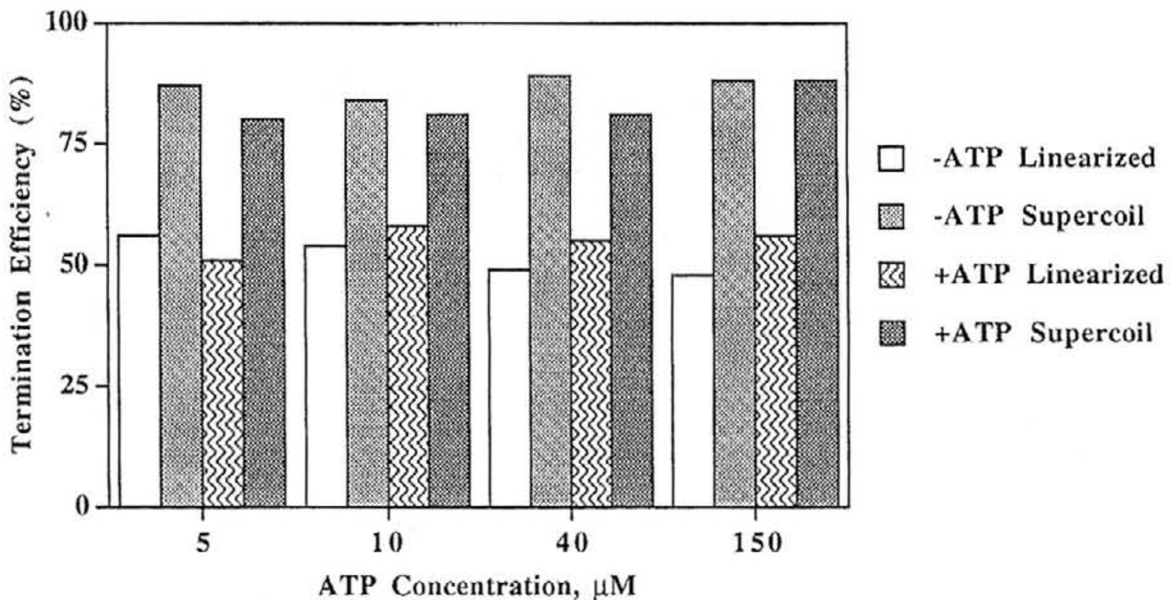


Fig. 5. Termination efficiencies of T7 RNA polymerase initiated by the different amounts of ATP. The transcription condition is described as in the legend of figure 4, and the termination efficiencies were determined as described in the text.

Richardson, 1986). T7 RNA polymerase recognizes the promoter of standard double-helical A- or B-form DNA, and binds mainly to one side of the DNA helix (Martin and Coleman, 1989). Compared to the absence of nucleoside triphosphate the protection region of T7 RNA polymerase in its promoter extends in the presence of only GTP (Gunderson *et al.*, 1987). This may imply that in the presence of GTP the conformation of T7 RNA polymerase may change. Here, T7 RNA polymerase and DNA template were preincubated with GTP, and T7 RNA polymerase may bind its promoter in a larger area than T7 RNA polymerase incubated with ATP did. Consequently, T7 RNA polymerase preincubated with GTP exhibited the efficient transcription producing much more RNA transcript than that preincubated with ATP (Figs. 3 and 6).

Promoter recognized by T7 RNA polymerase is a highly conserved sequence of 23 continuous bases, TAATACGACTCACTATAGGGAAA, within which the first G of the three continuous GGG is the first nucleotide of T7 RNA transcript (Mead *et al.*, 1986). Two conformational changes were revealed upon GTP binding to the lymerase-2-aminopurine DNA complex during the transcription performed by T7 RNA polymerase (Jia and Padel, 1997). Especially, the formation of the first two nucleotides, GG, is the rate limiting step in the transcription initiation of T7 RNA polymerase (Jia and Padel, 1997). Therefore, during the preincubation of T7 RNA polymerase with GTP, the formation of the first three nucleotides, GGG, was accomplished in this study. This may explain the preincubation of T7 RNA polymerase with GTP dramatically increased the production of RNA transcript (Fig. 3). Also, the carboxyl-terminus of T7 RNA polymerase was reported to interact with nucleotide (Gardner *et al.*, 1997). This may imply that the conformation of T7 RNA polymerase changes upon binding nucleotide.

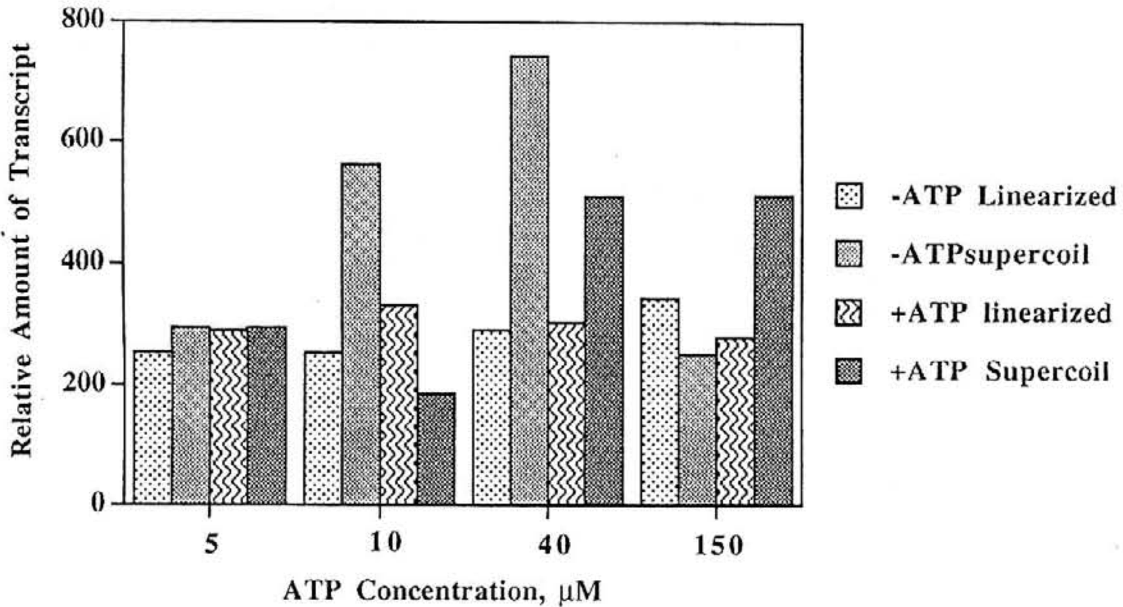


Fig. 6. Transcript production of T7 RNA polymerase initiated by the different amounts of ATP. The transcription condition is described as in the legend of figure 4, and the amount of transcript was determined as described in the text.

However, the various nucleotides may result in the different conformation of T7 RNA polymerase. The interaction between T7 RNA polymerase and nucleotides has been analyzed by fluorescence emission spectroscopy. The dissociation constant of GTP-T7 RNA polymerase is more stable than that of ATP-T7 RNA polymerase (Sen and Dasgupta, 1993). This result indicates that GTP has the higher affinity to T7 RNA polymerase than ATP does. Accordingly, during preincubation GTP affects T7 RNA polymerase profoundly, and results in the high efficiency of transcription (Fig. 3). Once the conformation of T7 RNA polymerase is altered due to the nucleotide binding, T7 RNA polymerase may remain its conformation during transcription. This may be the reason why the addition of four kind nucleotides, ATP, GTP, UTP, and CTP, at the same time did not enhance the transcription production by T7 RNA polymerase (reactions without preincubation in Figs. 3 and 6).

The transcription elongation and termination of *E. coli* RNA polymerase is also affected by the nucleotide concentrations. *E. coli* RNA polymerase was reported to pause at the specific region when the concentration of NTP was reduced (Fisher *et al.*, 1985; Kassaveit and Chamberlin, 1981; Reisbig and Hearst, 1981; Turnbough *et al.*, 1983). The pausing step is likely involved in the process of transcription termination (Farnham and Platt, 1980), so nucleotide concentrations affect the termination by *E. coli* RNA polymerase (Reynolds, 1988). Bacteriophage T7 RNA polymerases always show higher termination efficiencies when the concentrations of NTPs is lowered (Jeng and Lee, 1998). It seems that the pausing step also occurs in the termination process of T7 RNA polymerase. However, the similar result was not obtained in this report, and probably the nucleotide concentration is not low enough to show such effect.

Most DNA within cell is supercoiled form, which is regulated by DNA gyrase and topoisomerase I (DiNardo *at al.*, 1982). The transcription efficiency of *E. coli* RNA

polymerase was significantly affected by the topology of DNA (Leory and Wang, 1987). The stability of T7 RNA polymerase on its promoter at the initiation complex was at least 14 times more stable in the supercoiled DNA template than that in the relaxed DNA (Diaz *et al.*, 1996). Previously, we found that the transcription efficiency of T7 RNA polymerase in the supercoiled DNA was better than in the linearized DNA template (Jeng and Lee, 1998). Here, compared to the linearized DNA template the production of RNA transcript was increased in the supercoiled DNA template (Figs. 3 and 6). It may indicate that T7 RNA polymerase has the high affinity to supercoiled promoter, and favors the supercoiled DNA as template to perform transcription.

## ACKNOWLEDGEMENTS

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# 在不同核酸形式下 GTP 和 ATP 對於噬菌體 T7 核醣核酸聚合酶轉錄的影響

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## 摘　　要

在不同核酸形式下分析 GTP 和 ATP 對於噬菌體 T7 核醣核酸聚合酶轉錄終止和核酸產量的影響。T7 核醣核酸聚合酶和帶有酥氨酸衰減子的不同形式核酸模板先和 GTP 或 ATP 作用，再加入四種核甘酸以啟動轉錄反應。結果發現在超螺旋狀核酸模板上，T7 核醣核酸聚合酶對於酥氨酸衰減子所產生的轉錄終止效率，較在線形核酸模板上為高。並且 T7 核醣核酸聚合酶先和 GTP 作用者，在超螺旋狀核酸模板上所產生的核酸產量，較在線形核酸模板上高出 4-21 倍。同樣在超螺旋狀核酸模板上，T7 核醣核酸聚合酶先和 GTP 作用者，所產生的核酸產量，較沒有先和 GTP 作用者多出 3.6-5.0 倍。這個結果顯示不同核酸形式會影響 T7 核醣核酸聚合酶的核酸產量，並且 GTP 會改變 T7 核醣核酸聚合酶在轉錄啟始時的構造。

關鍵詞：噬菌體 T7 RNA 聚合酶、轉錄終止、超螺旋狀去氧核醣核酸。

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