THEORETICAL ANALYSIS OF SINGLE-ROUND TRANSCRIPTION EXPERIMENTS ON *trp* LEADER REGION

HIROMI SUZUKI, TAKASHI KUNISAWA, AND JINYA OTSUKA
Department of Applied Biological Science, Faculty of Science and Technology, Science University of Tokyo, Noda (278), Japan

ABSTRACT A kinetic model is proposed to reproduce the time courses of the concentration change in paused leader RNA, terminated leader RNA, and readthrough RNA in the single-round transcription experiments on *trp* leader region of *Escherichia coli* and its mutants, L132, L75, and L75L135 (Winkler, M. E., and C. Yanofsky, 1981, *Biochemistry*, 20:3738–3744; Fisher, R., and C. Yanofsky, 1983, *J. Biol. Chem.*, 258:9208–9212). This model fits the experimental results well and also captures the essential aspects of the processes of transcriptional pausing and termination. In the wild type template, under optimal conditions, it is found that the transcription rate at the pause and attenuation sites is of the same order of magnitude, 10,000-fold lower than the transcription rate at the other sites, and the high termination level at the attenuation site is attributable to a higher dissociation rate. This analysis also provides a clue as to how the template base change, various concentrations of ribonucleoside triphosphates, and the presence or absence of *L*-factor affect the transcription and dissociation rates to yield different termination levels at the pause or attenuation site. It also discusses the molecular mechanism of the transcriptional pausing and termination.

INTRODUCTION

Termination of transcription is known to play an important role in the regulation of gene expression. In most cases, RNA polymerase terminates transcription efficiently at the end of genes. In some cases, however, partial termination controls the transcription of the downstream structural genes. In particular, the transcription of most biosynthetic operons of amino acids in enteric bacteria is under attenuation control in the leader region through coupling with translation (Lee and Yanofsky, 1977; Zurawski et al., 1978; Oxender et al., 1979). It has also been shown that the RNA polymerase molecule pauses during the transcription of the leader region (Winkler and Yanofsky, 1981). In assessing the attenuation control mechanism, including the effect of transcriptional pausing, it is necessary to evaluate the transcription rate at the pause and attenuation sites as well as at the other sites since attenuation control efficiency depends strictly on the position of RNA polymerase on the template. As pointed out previously (Suzuki et al., 1986), this efficiency is evaluated by the probability that RNA polymerase arrives at the attenuation site while ribosome stays at the "own" codon(s).

Studies on base sequence pattern in the attenuation and termination sites have revealed the common feature that the DNA sequence immediately preceding these sites is comprised of AT base pairs corresponding to a series of uridine residues in the 3'-terminus of the transcript following a GC-rich dyad symmetry sequence (Rosenberg and Court, 1979; Otsuka and Kunisawa, 1982). In the tryptophan operon of *E. coli*, partial termination of transcription occurs at the attenuation site in vivo (Bertrand et al., 1976), but in vitro the attenuator functions as an efficient terminator without added factors. The attenuator is thus considered to be a model system for termination of transcription (Bertrand et al., 1975; Lee and Yanofsky, 1977; Fuller and Platt, 1978). The base sequence with GC-rich dyad symmetry is also recognized in the region preceding the pause site, but the intervening sequence between the palindrome and the pause site is not AT-rich (Winkler and Yanofsky, 1981).

In this paper, an attempt is made to evaluate the transcription rate and dissociation rate of transcripts at the pause and attenuation sites by use of the data presented in the single-round transcription experiments carried out in vitro on *E. coli trp* leader region by Winkler and Yanofsky (1981) and Fisher and Yanofsky (1983). Although the termination mechanism has so far been discussed only in terms of the termination level, the present analysis also provides us with several cases that examine how the template base change affects the transcription and dissociation rates at the pause or attenuation site to yield different termination levels.

METHODS

The template DNAs used in the single-round transcription experiments (Winkler and Yanofsky, 1981; Fisher and Yanofsky, 1983) are shown in Fig. 1, in which replaced bases in three mutants, L132, L75, and L75L135, are also indicated. In the single-round transcription experiments, RNA polymerase is added to a standard reaction mixture that contains only ATP and GTP as ribonucleoside triphosphates and template DNA. The reaction allows the formation of an initiation complex that consists of RNA polymerase, template DNA, and the transcript containing the first three nucleotides, i.e., AAG (see Fig. 1). Single-round

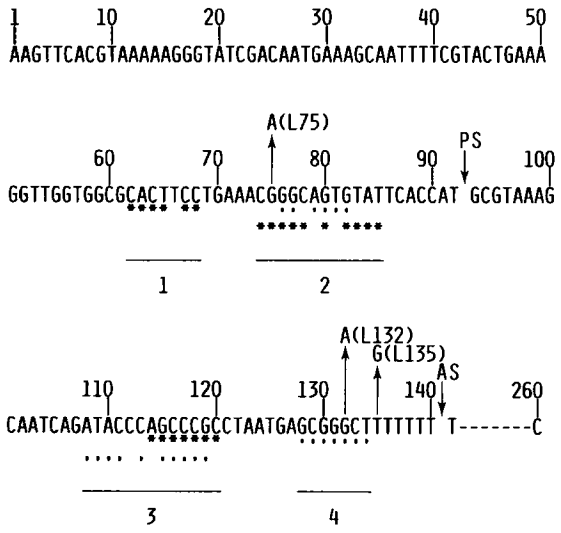
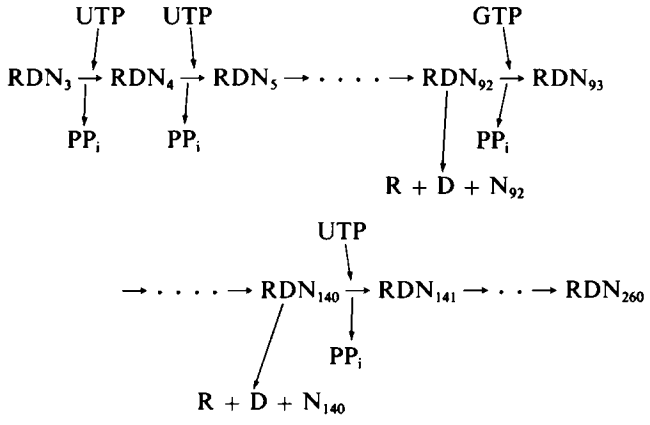


FIGURE 1 DNA sequence of the *trp* leader and attenuation regions of *E. coli*. PS, pause site of RNA polymerase molecule; AS, attenuation site of transcription. The DNA bases complementary to each other in the palindrome are denoted by * and ■. The formation of the following three types of hairpin loop structures is possible in the transcript; 1 · 2 stem and loop, 2 · 3 stem and loop, and 3 · 4 stem and loop (Oxender et al., 1979). The base changes in the *trp*L75, *trp*L132 and *trp*L75L135 mutants are also shown by arrows.

transcription is then started by addition of the prewarmed solution containing CTP, UTP, and rifampicin or heparin, which prevents subsequent rounds of transcription initiation.

This procedure of single-round transcription enables us to describe the following kinetic scheme:



Scheme I

where R, D, N_i, and RDN_i represent RNA polymerase, template DNA, transcript after incorporation of ith ribonucleoside triphosphate, and RNA polymerase–template–transcript complex after incorporation of the ith ribonucleoside triphosphate. It is well known that RNA polymerase either terminates transcription by producing a 140 nucleotide-long transcript or continues to transcribe further bases in the downstream region (Bertrand et al., 1976). As for the pause site, major paused RNA has now been identified as being 92 nucleotides long (Fisher and Yanofsky, 1983), although it had been reported to be 91 nucleotides long (Winkler and Yanofsky, 1981). As shown in a later section, most of the experimental data are well reproduced if some of the transcripts are released even at the pause site. Since the single-round transcription consumes no more than 10^{-3}% of the initial concentration of any of four kinds of ribonucleoside triphosphates, the ribonucleoside triphosphate concentrations can be regarded as time-independent. The transcription rate may depend strictly on the ribonucleoside triphosphate species. Furthermore, concentrations are not set to be the same for the four ribonucleoside triphosphates in the single-round transcription experiments. For the present analysis, however, a common incorporation rate is assumed independently of the ribonucleoside triphosphate species since the four nucleotides appear with almost equal frequency in such long chains as those containing 92, 140, and 260 nucleotides. In this assumption, the following set of differential equations is evolved:

$$
\begin{cases}
\dfrac{d}{dt}[RDN_3] = -\lambda^*[RDN_3], \\
\dfrac{d}{dt}[RDN_4] = \lambda^*[RDN_3] - \lambda[RDN_4], \\
\quad\vdots \\
\dfrac{d}{dt}[RDN_{92}] = \lambda[RDN_{91}] - \lambda_p[RDN_{92}] - \nu_p[RDN_{92}], \\
\dfrac{d}{dt}[RDN_{93}] = \lambda_p[RDN_{92}] - \lambda[RDN_{93}], \\
\quad\vdots \\
\dfrac{d}{dt}[RDN_{140}] = \lambda[RDN_{139}] - \lambda_a[RDN_{140}] - \nu_a[RDN_{140}], \\
\dfrac{d}{dt}[RDN_{141}] = \lambda_a[RDN_{140}] - \lambda[RDN_{141}], \\
\quad\vdots \\
\dfrac{d}{dt}[RDN_{260}] = \lambda[RDN_{259}], \\
\dfrac{d}{dt}[N_{92}] = \nu_p[RDN_{92}], \\
\dfrac{d}{dt}[N_{140}] = \nu_a[RDN_{140}],
\end{cases}
\tag{1}
$$

where the transcription rates, λ^* of the initiation complex, λ_p at the pause site and λ_a at the attenuation site, are distinguished from the transcription rate λ at the other sites. The dissociation rates of the transcripts at pause and attenuation sites are denoted by ν_p and ν_a, respectively. With the initial condition that only RDN_3 is present at the time $t = 0$, this set of equations is easily integrated with respect to time t. The quantities $[RDN_{92}] + [N_{92}]$, $[RDN_{140}] + [N_{140}]$ and $[RDN_{260}]$, expressed as functions of time t, are compared with the concentrations of paused leader (PL) RNA, terminated leader (TL) RNA, and readthrough (RT) RNA, respectively, in the single-round transcription experiments. These quantities are calculated with the use of sets of six parameters, λ, λ^*, λ_p, λ_a ν_p, and ν_a, and are plotted against time t.

According to the set of differential equations (Eq. 1), the transcription rate λ^* of initiation complex is evaluated to reproduce the relation between the initial slope and the peak of PL-RNA curve. Eq. 1 also suggests the following properties. The PL-RNA curve decreases approximately with a decay factor of λ_p after the peak and finally approaches $[RDN_3(0)]\ \nu_p/(\lambda_p + \nu_p)$, where $[RDN_3(0)]$ is the initial amount of initiation complex. The TL-RNA curve finally approaches $[RDN_3(0)]\ \lambda_p\nu_a/(\lambda_p + \nu_p)(\lambda_a + \nu_a)$. The final ratio of TL-RNA and RT-RNA becomes ν_a/λ_a. From these properties, the values of λ_p, ν_p, λ_a, and ν_a can be

estimated preliminarily. These estimated values are further refined by the method of least squares, although some data that seem to have large experimental errors are disregarded in this procedure.

RESULTS

The values of transcription rates (λ, λ^*, λ_p, and λ_a) and of dissociation rates (ν_p and ν_a) used in the present curve fittings are listed in Table I, together with the quantities $\nu_p/(\lambda_p + \nu_p)$ and $\nu_a/(\lambda_a + \nu_a)$, which correspond to the transcription termination levels at the pause and attenuation sites, respectively. As shown in Figs. 2, *a–d*, 3, *a* and *b*, and 4, the calculated curves of $[RDN_{92}] + [N_{92}]$, $[RDN_{140}] + [N_{140}]$ and $[RDN_{260}]$ reproduce fairly well the concentration change with time in PL-, TL-, and RT-RNAs. As seen from Table I, the transcription rates λ and λ^* mainly depend on the experimental conditions such as concentrations of ribonucleoside triphosphates, temperature, and the presence or absence of *L*-factor, whereas the transcription and dissociation rates at the pause and attenuation sites depend on both experimental condition and the template. The small value of λ^* implies that RNA polymerase in the initiation complex may be trapped in a form different from the usual elongation one.

The experimental data (condition *i*), shown in Fig. 2, are obtained at ribonucleoside triphosphate concentrations different from those in Fig. 3 (condition *ii*). By an increase in the concentrations of ribonucleoside triphosphates, the transcription rate λ in the latter condition is assessed to be 1.5-fold higher than in the former case. The transcription rate λ^* of initiation complex also increases twofold in the latter case, reflecting a 2.7-fold higher concentration of UTP, which is incorporated into the transcript as the fourth nucleotide. Under the experimental condition shown in Fig. 2, a–d, the transcription rate λ_p at the pause site is evaluated to be extremely low, and some of the transcripts are dissociated even from the pause site in the wild type template. This may be due to the low concentration of GTP, which is the first ribonucleoside triphosphate to be incorporated after the pausing. In contrast, the PL-RNA

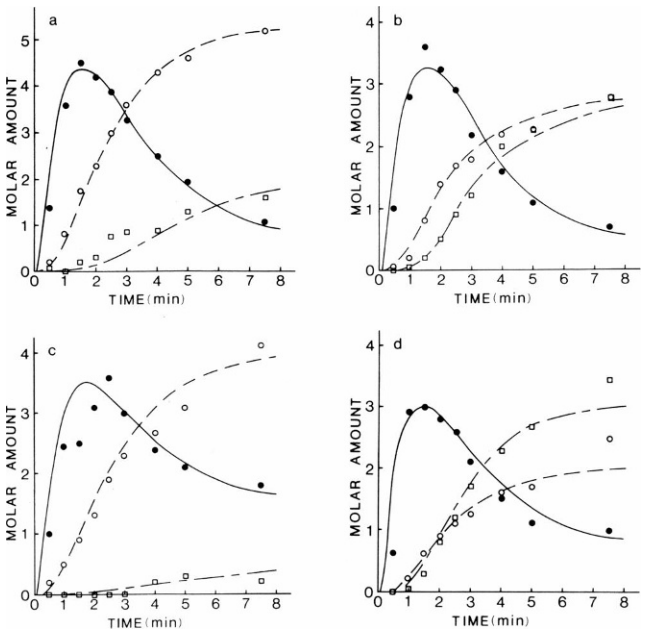


FIGURE 2 Comparison of calculated time course of RNA synthesis with the experimental data (Winkler and Yanofsky, 1981) obtained at 22°C and at ribonucleoside triphosphate concentrations; 20 μM GTP and 150 μM each of ATP, CTP, and UTP (condition *i*). Restriction fragments used as templates in this experiment were from wild-type (*a*), mutant *trp*L132 (*b*), mutant *trp*L75 (*c*), and mutant *trp*L75L135 (*d*). The symbols ●, O, and □ denote experimental values for PL-RNA, TL-RNA and RT-RNA, respectively. The lines —, -----, and ——— denote calculated curves for $[RDN_{92}] + [N_{92}]$, $[RDN_{140}] + [N_{140}]$, and $[RDN_{260}]$, respectivley. Multiplication of ordinate values by 3×10^{-17} gives actual molar amounts.

almost disappears within 3 min, in the experimental data in Fig. 3. These data are obtained under the condition of a much higher concentration of GTP. The evaluated value of transcription rate λ_p is only 200–300-fold smaller than that of the transcription rate λ at other sites. Thus, most of the transcripts have continued to be elongated, even if the dissociation rate at the pause site is of the same order of magnitude as in the experiment shown in Fig. 2. It may

TABLE I
TRANSCRIPTION RATES AND DISSOCIATION RATES TO REPRODUCE DATA IN FIGS. 2–4

Condition	Template	λ	λ^*	λ_p	λ_a	ν_p	ν_a	$\nu_p/(\lambda_p + \nu_p)$	$\nu_a/(\lambda_a + \nu_a)$
i	Wild-type	600	1.10	0.42	0.15	0.03	0.35	0.07	0.70
	*trp*L132	600	1.10	0.44	1.05	0.02	1.05	0.04	0.50
	*trp*L75	600	1.10	0.40	0.05	0.13	0.40	0.25	0.89
	*trp*L75L135	600	1.10	0.50	3.70	0.07	2.30	0.12	0.38
ii	Wild-type	880	2.10	3.00	0.52	0 ~ 0.04	1.08	~0	0.68
	*trp*L75L135	880	2.10	3.80	6.00	0 ~ 0.04	2.00	~0	0.25
iii	Wild-type	2,700	0.97	0.89	0.30	0.05	9.60	0.05	0.97

λ, λ^*, λ_p, λ_a, transcription rates at nonspecific, initiation, pause, and attenuation sites, respectively (nucleotide · min^{-1}).
ν_p, ν_a, dissociation rates at pause and attenuation sites (min^{-1}).
Condition *i:* 20 μM GTP, 150 μM each of ATP, CTP, and UTP at 22°C.
Condition *ii:* 30 μM CTP, 150 μM ATP, 150 μM GTP, and 400 μM UTP at 22°C.
Condition *iii:* 20 μM GTP, 150 μM each of ATP, CTP, and UTP at 37°C in the presence of *L*-factor.

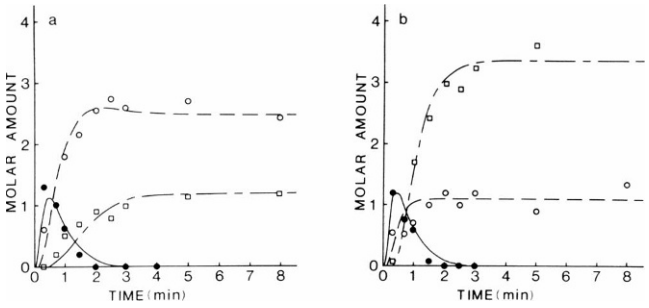


FIGURE 3 Comparison of calculated time course of RNA synthesis with the experimental data (Winkler and Yanofsky, 1981) on the restriction fragments from wild type (*a*) and mutant *trp*L75L135 (*b*) at 22°C and at ribonucleoside triphosphate concentrations; 30 μM CTP, 150 μM ATP, 150 μM GTP, and 400 μM UTP (condition *ii*). The symbols and the lines used herein denote the same meanings as in Fig. 2. Multiplication of ordinate values by 4.4×10^{-17} gives actual molar amounts.

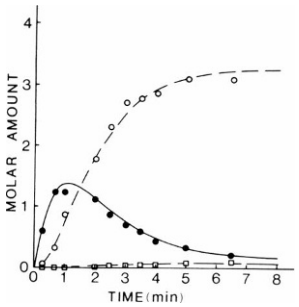


FIGURE 4 Comparison of calculated time course of RNA synthesis with the experimental data on the wild type of template by Fisher and Yanofsky (1983). The experiment was carried out at 37°C and in the presence of *L*-factor at ribonucleoside triphosphate concentrations; 20 μM GTP and 150 μM each of ATP, CTP, and UTP (condition *iii*). The same symbols and lines as in Fig. 2 are used herein. Multiplication of ordinate values by 4.5×10^{-15} gives actual molar amounts.

also be due to the difference in UTP concentration that the transcription rate λ_a at the attenuation site increases under condition *ii* rather than under condition *i*.

The present analysis also reproduces the experimental data in the presence of *L*-factor, as shown in Fig. 4. This factor is known to enhance transcriptional pausing (Farnham et al., 1982; Fisher and Yanofsky, 1983). Since the concentrations of ribonucleoside triphosphates in this experiment are the same as all those in condition *i*, the 4.5-fold higher value of λ in this condition may be due to the higher temperature of 37°C. Conversely, both λ_p and λ_a are only twofold higher, and λ^* is suppressed to almost the same value as obtained at 22°C. This result shows that *L*-factor lowers the transcription rate λ^* of the initiation complex as well as the transcription rates at the pause and attenuation sites. The dissociation rate ν_a at the attenuation site is shown to greatly increase in value while the dissociation rate ν_p at the pause site remains low. This may be due to the fact that the weak hydrogen bonds between U and A bases, which are easily broken at higher temperature, are abundant in the transcript–template complex at the attenuation site. It is worth noting that the dissociation of the transcript is prohibited at the pause site and most of the transcripts are released from the attenuation site under the optimal conditions of the presence of *L*-factor and the higher temperature.

The base replacement in the template DNA also affects the transcription and dissociation rates at the pause and attenuation sites. In the mutant L132, the replacement of a GC base pair in the palindrome of the attenuation region by an AT base pair causes the transcription and dissociation rates to increase and reduces the termination level at the attenuation site. Since it is inconceivable that the replacement of downstream base G(132) → A(132) influences the time course of PL-RNA, the curve fitting to this RNA species is carried out in disregard of an experimental value of PL-RNA between 1 and 2 min (cf. Fig. 2 *b*). In practice, most of the other data from mutant L132 PL-RNA are hardly distinguishable from those of the wild type PL-RNA. In mutant L75, the dyad symmetry of the palindromic sequence in the pause region becomes more complete than in wild type. This base change is shown to influence the termination level at both the pause and attenuation sites. At the pause site, the transcription rate λ_p remains almost the same value as in the wild type, but the dissociation rate is increased ~fourfold compared with the wild type. As seen in Fig. 2 *c*, the experimental data, especially of PL-RNA, on this mutant appear to be insufficient. If we fit only the data of PL-RNA using the method of least squares, a smaller value of λ and larger values of λ_p and ν_p are obtained. These parameter values, however, give rise to a greater deviation from the experimental data of the time courses of TL- and RT-RNAs, even if any values of λ_a and ν_a are used. The enhanced termination level at the attenuation site may be interpreted by the mechanism that higher probability of 1 · 2 stem and loop formation facilitates the formation of 3 · 4 stem and loop structure in the leader transcript (Oxender et al., 1979). According to the present analysis, this enhanced termination level results from the fact that the transcription rate λ_a is decreased to one-third of that in the wild type while the dissociation rate remains almost equal to that in wild type. In mutant L75L135, however, both transcription rate and dissociation rate at the attenuation site are greatly increased to yield the low termination level, although base replacement T(135) → G(135) seems to strengthen the stability of 3 · 4 stem and loop structure in the transcript. The low termination level at the attenuation site of mutant L75L135 is also recognized in the experimental data, as shown in Fig. 3, where wild type and mutant L75L135 are compared. Furthermore, the termination level at the pause site, which is increased to 25% in mutant L75, seems to be slightly diminished in mutant L75L135. These effects of template base changes on the transcription and dissociation rates will be discussed in the last section.

DISCUSSION AND CONCLUSION

The present analysis shows that the time courses of the concentration change in PL-, TL- and RT-RNAs can be well reproduced by Eq. 1. Although a trial to estimate the dependency of transcription rate on nucleotide species has been attempted (Aivazashvili et al., 1981), the result of this attempt is not simple; the transcription rate of any of the four nucleotide species depends on the neighboring nucleotides, and the synthesis rate of trinucleotides is only classified semi-quantitatively into three ranks. Therefore, it is not easy to take account of the difference in transcription rate between nucleotide species in the calculation of time courses of PL-, TL-, and RT-RNAs. Nevertheless, the present results show that the transcription rates at ordinary sites in long chains such as those 92, 140, and 260 nucleotides in length are averaged to a common transcription rate λ if the transcription rate and dissociation rate at the pause and attenuation sites are suitably chosen.

According to the present analysis, the transcription rate at the pause and attenuation sites is 10,000-fold lower than that at other sites and most of the transcripts are released at the attenuation site under optimal conditions, as long as the wild type DNA is used as the template. This reconfirms a model consideration holding that RNA polymerase transcribing the leader region only pauses at the pause site without dissociation of transcript and the transcription termination occurs completely at the attenuation site unless ribosome prevents the transcript from forming the 3 · 4 stem and loop structure (Oxender et al., 1979; Winkler and Yanofsky, 1981). This model consideration, together with the much lower transcription rate at the pause site, points to an important role for the pause site in the attenuation control mechanism; i.e., the transcriptional pausing makes the attenuation control more sensitive as an on/off switch for the concentration of tryptophan or of tRNA charged with this amino acid and diminishes the dependence of attenuation control characteristics on the number of nucleotides between the ribosome binding site, own codons, and the attenuation site (Suzuki et al., 1986). It should be noted, however, that partial releasing of transcripts at the pause site is recognized under some conditions. Since the transcription rate at the pause site is of almost the same order of magnitude as that at the attenuation site the dissociation rate, the termination levels at these sites are easily changed depending on the experimental conditions such as varying concentrations of ribonucleoside triphosphates, temperature, and the presence or absence of *L*-factor.

The mechanism of transcription termination has been discussed mainly in the comparison of the termination level with base sequence pattern in the template or transcript. The present results suggest that the termination mechanism is preferably discussed in terms of the transcription and dissociation rates. Most of the termination levels evaluated in the present analysis, except for that of mutant L75L135, are consistent with the usual interpretation that the formation of a more stable hairpin loop structure in the transcript results in a higher termination level (Rosenberg et al., 1978; Rosenberg and Court, 1979; Oxender et al., 1979). However, this does not necessarily mean that the formation of a more stable hairpin loop structure results in the decrease of transcription rate and the increase of dissociation rate. The comparison between the pause site and attenuation site suggests the tendency of the palindrome with higher symmetry or the formation of a more stable hairpin loop structure in the transcript to decrease the transcription rate and of more AT pairs to increase the dissociation rate. This tendency is also recognized in mutants L132 and L75. The experimental studies on the *trp* attenuator using base analog (Farnham and Platt, 1980) also suggest both RNA–RNA and RNA–DNA interactions, although the transcription and dissociation rates at the attenuation site cannot be estimated separately from these experimental data. At any rate, further studies seem to be needed to elucidate the response of RNA polymerase at the pause or attenuation site. First, the experimental data available at the present time are too limited, and some of them may not be sufficiently reliable to allow meaningful quantitative parameter fitting. For example, the difference in transcription and dissociation rates at the pause site between the mutants L75 and L75L135 is only slight. Second, the slight difference in transcription rate produced by one base replacement might be caused not only by the change in the stability of hairpin loop structure but also by the difference in transcription rate intrinsic to the nucleotide species. In practice, the synthesis rate of UUU belongs to the lowest rank according to the study by Aivazashvili et al. (1981), and this might help explain the high transcription rate at the attenuation site in mutant L75L135. Thus, it seems desirable to construct a series of template DNAs in which the nucleotide bases in the palindrome and AT base pairs are successively replaced by other bases. The single-round transcription measurements on these templates, ranging from partial to complete disruption of palindrome or poly AT base pairs, and theoretical analyses of them are expected to reveal a clearer relation of the transcription rate and dissociation rate to the base sequence pattern.

We thank Prof. K. Satoh for reading the manuscript and for valuable suggestions.

Received for publication 30 June 1986 and in final form 2 October 1986.

REFERENCES

Aivazashvili, V. A., R. Sh. Bibilashvili, R. M. Vartikyan, and T. A. Kutateladze. 1981. Relationship between the primary RNA structure

and pulse elongation *in vitro* by *Escherichia coli* RNA polymerase. A model. *Molekulyarnaya Biologiya.* 15:915–929.

Bertrand, K., L. Korn, F. Lee, T. Platt, C. L. Squires, C. Squires, and C. Yanofsky. 1975. New features of the regulation of the tryptophan operon. *Science (Wash. DC).* 189:22–26.

Bertrand, K., C. Squires, and C. Yanofsky. 1976. Transcription termination *in vivo* in the leader region of the tryptophan operon of *Escherichia coli. J. Mol. Biol.* 103:319–337.

Farnham, P. J., and T. Platt. 1980. A model for transcription termination suggested by studies on the *trp* attenuator *in vitro* using base analogs. *Cell.* 20:739–748.

Farnham, P. J., J. Greenblatt, and T. Platt. 1982. Effects of *nusA* protein on transcription termination in the tryptophan operon of *Escherichia coli. Cell.* 29:945–951.

Fisher, R., and C. Yanofsky. 1983. A complementary DNA oligomer releases a transcription pause complex. *J. Biol. Chem.* 258:9208–9212.

Fuller, R. S., and T. Platt. 1978. The attenuator of the tryptophan operon in *E. coli:* rho-mediated release of RNA polymerase from a transcription termination complex *in vitro. Nucleic Acids Res.* 5:4613–4623.

Lee, F., and C. Yanofsky. 1977. Transcription termination at the *trp* operon attenuators of *Escherichia coli* and *Salmonella typhimurium:* RNA secondary structure and regulation of termination. *Proc. Natl. Acad. Sci. USA.* 74:4365–4369.

Otsuka, J., and T. Kunisawa. 1982. Characteristic base sequence patterns of promoter and terminator sites in ϕx174 and fd phage DNAs. *J. Theor. Biol.* 97:415–436.

Oxender, D. L., G. Zurawski, and C. Yanofsky. 1979. Attenuation in the *Escherichia coli* tryptophan operon: role of RNA secondary structure involving the tryptophan codon region. *Proc. Natl. Acad. Sci. USA.* 76:5524–5528.

Rosenberg, M., D. Court, D. L. Wulff, H. Shimatake, and C. Brady. 1978. The regulation between function and DNA sequence in an intercistronic regulatory region in phage λ. *Nature (Lond.)* 272:414–423.

Rosenberg, M., and D. Court. 1979. Regulatory sequences involved in the promotion and termination of RNA transcription. *Annu. Rev. Genet.* 13:319–353.

Suzuki, H., T. Kunisawa, and J. Otsuka. 1986. Theoretical evaluation of transcriptional pausing effect on the attenuation in *trp* leader sequence. *Biophys. J.* 49:425–436.

Winkler, M. E., and C. Yanofsky. 1981. Pausing of RNA polymerase during *in vitro* transcription of the tryptophan operon leader region. *Biochemistry.* 20:3738–3744.

Zurawski, G., D. Elseviers, G. V. Stauffer, and C. Yanofsky. 1978. Translational control of transcription termination at the attenuator of the *Escherichia coli* tryptophan operon. *Proc. Natl. Acad. Sci. USA.* 75:5988–5992.