JOURNAL OF BACTERIOLOGY, Oct. 1992, p. 6071–6075
0021-9193/92/196071-05$02.00/0
Copyright © 1992, American Society for Microbiology
Vol. 174, No. 19

# Melting during Steady-State Transcription of the *rrnB* $P_1$ Promoter In Vivo and In Vitro

KARI L. OHLSEN† AND JAY D. GRALLA*

*University of California at Los Angeles and The Molecular Biology Institute, Los Angeles, California 90024-1569*

Received 3 March 1992/Accepted 30 July 1992

**The rRNA *rrnB* $P_1$ promoter was probed with the single-strand-selective reagent potassium permanganate during steady-state transcription in vitro and in vivo. In both cases, a weak but significant level of permanganate sensitivity was observed, which was not changed by treatment with rifampin. In contrast, static studies showed that rifampin strongly affects the very high level signal associated with polymerases that have used ATP and CTP as initiating nucleotides. We infer that the permanganate sensitivity associated with steady-state transcription is due to polymerases that have not yet used ATP and CTP. The slow and regulated step during *rrnB* $P_1$ transcription may be the use of the initiating nucleotides to catalyze stable opening of the promoter DNA.**

When *Escherichia coli* is subjected to conditions of carbon or nitrogen starvation, intracellular concentrations of the signal guanosine tetraphosphate (ppGpp) increase dramatically (15), a process which in turn is correlated with alterations in the transcriptional profile of the stressed cell (for a review, see reference 7). The rRNA operons in particular are strongly inhibited by starvation, which underscores their central metabolic role in cellular growth. The promoters that direct the synthesis of rRNA have been studied extensively in vitro and in plasmid systems in vivo. Many studies have shown that these promoters can be inhibited by ppGpp, but the molecular step at which the inhibition of transcription occurs remains uncertain.

In principle, transcription from a promoter may be rate limited at any of several steps (10, 18, 28). The most prominent of these are formation of a closed complex, melting of this complex to form an open complex, and conversion of the open complex to an elongation complex. Previously, we have obtained information about the in vivo rate limitation at the *lac* promoter (28). In those experiments, melted DNA was assayed in vivo by permanganate sensitivity. The amount of melting during steady-state transcription was compared with the amount detected when the cells are pretreated with rifampin, which, by blocking initiation, allows uninitiated open complexes to accumulate. For the *lac* and $glnAp_2$ promoters, the signal is much smaller without rifampin than with the drug (27, 28). This finding suggests that polymerases are not generally poised at the promoters in open complexes during transcription, since if they were, the signal would not increase with rifampin treatment. We now wish to extend such studies to the *rrnB* $P_1$ promoter.

There are several problems associated with probing the ribosomal promoters in vivo. There are seven very similar copies of the ribosomal operons in vivo, which complicates detecting the state of any one. We have overcome this problem by using a primer for detection of the chromosomal copy of the gene that is complementary to a unique sequence upstream from the *rrnB* $P_1$ promoter. Another complicating factor relates to the requirement for the initiating nucleotides ATP and CTP for heparin-resistant complex formation at *rrnB* $P_1$ (9, 19). This unusual property may affect the promoter response to rifampin. Rifampin inhibits initiation of transcription while leaving elongating polymerases unaffected (29) and can thus be used to trap polymerase prior to elongation. This inhibition has been shown to be somewhat effective during formation of the first dinucleotide bond and very inhibitory during formation of the second dinucleotide bond (4, 11). Thus, it is uncertain what the effect of rifampin will be at *rrnB* $P_1$, since formation of the first bond is required to form open complexes efficiently. Therefore, we began these experiments by assaying the effects of rifampin on complex formation at this promoter.

## MATERIALS AND METHODS

Plasmid pKK3535 was obtained from H. Noller (2). RNA polymerase was prepared by C. W. Wong in this laboratory according to the method of Burgess and Jendrisak (3). The amounts used in these experiments are specified below. Strain MC1000 [*araD139 thiA* Δ(*ara, leu*)*7697* Δ*lacX74 galU galK strA*] was from R. C. Johnson.

**In vitro complex formation and elongation conditions.** The template used in these experiments was plasmid pKK3535, which contains a 7,506-bp insert containing both the $P_1$ and $P_2$ promoters as well as the *rrnB* structural genes and a 4,363-bp fragment containing the pBR322 vector sequences. One microgram of the 12-kb supercoiled plasmid DNA was incubated in 10 μl of 1× transcription salts (3 mM $MgCl_2$, 0.2 mM dithiothreitol, 0.1 mM EDTA, 30 mM Tris [pH 8.0], 100 mM KCl). ATP (500 μM) and CTP (100 μM) were contained in the mixture except in the case of reaction mixtures lacking nucleotide, to which they were added in the same quantities at a later step (see legend to Fig. 1). RNA polymerase was diluted serially on ice in transcription salts containing 200 μg of bovine serum albumin per ml and 5% glycerol. The DNA was prewarmed for 5 min at 37°C, and then 10 μl of the diluted RNA polymerase which had been prewarmed for 1.5 min was added to make a 20-μl reaction volume. Incubations were carried out for 10 min. When desired, elongation was allowed to occur by the addition of 100 μM GTP and 100 μM

* Corresponding author.
† Present address: Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, CA 92037.

UTP for 10 min. When present, 1 μl of 4-mg/ml rifampin was added, as described in the figure legends. In these experiments, a nominal RNA polymerase concentration of 250 nM was used, which was determined to be twice that needed for saturation of the promoters.

Steady-state transcription conditions were established on supercoiled DNA by the addition of a nominal concentration of 625 nM RNA polymerase to a mixture of transcription salts, 500 μM ATP, and 100 μM CTP. After a 10-min incubation, 100 μM GTP and 100 μM UTP were added to allow elongation for up to 4 min. When present, 1 μl of 4-mg/ml rifampin or 1 μl of 6 mM ppGpp was added halfway through this incubation, allowing elongation for a further 10 min before footprinting. The ppGpp used in these experiments was confirmed to be intact by thin-layer chromatography and to be active at inhibition of transcription by runoff transcription assays.

**In vitro footprinting.** After incubation, the mixture was treated with 0.5 μl of 0.37 M $KMnO_4$ for 15 s. The reaction was then quenched by addition of 2.0 μl of 14.7 M β-mercaptoethanol (neat), and DNA was purified as previously described (28).

**In vivo footprinting.** Cells were grown to mid-log phase (optical density of ~0.5) in glucose minimal medium prepared with M9 salts containing 5 μg of thiamine per ml, 0.2% glucose, and 5 mg of Casamino Acids per ml. When present, 40 μl of 50-mg/ml rifampin was added to 10 ml of culture 5 min prior to addition of 270 μl of 0.37 M $KMnO_4$ for a 2-min reaction. Chromosomal DNA was harvested by the method of Owen and Borman (23) and purified as described previously (26, 28). The final volume of DNA solution after passage through the spin column was brought to 100 μl.

**Primer extension.** Primer extension was carried out with an end-labeled primer which hybridizes nucleotides −87 to −70 from the start site and reads downstream into the promoters. Alkaline denaturation conditions were used, followed by hybridization and Klenow extension at 50°C (26). Approximately 30 μl of chromosomal DNA and 500,000 cpm of labeled primer were used for each lane.

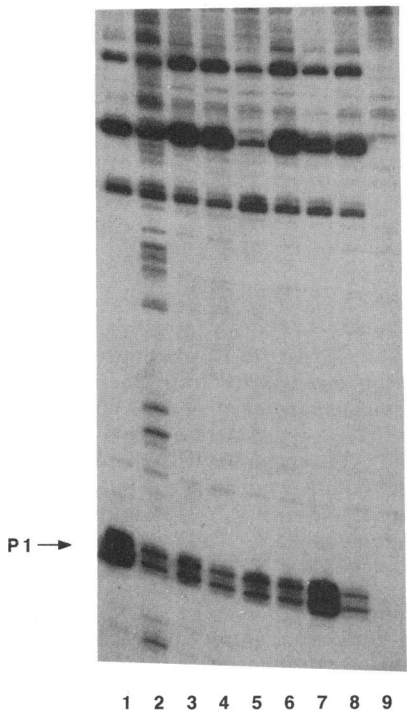


FIG. 1. Effects of rifampin and nucleotides on melting, determined by $KMnO_4$ probing on complexes with supercoiled DNA. Complex formation reaction mixtures contained ATP and CTP except in lanes 5 and 6. In lanes 2, 4, 6, and 8, GTP and UTP were added after complex formation to promote elongation. Rifampin was added as follows: lanes 1 and 2, none added; lanes 3 and 4, added to polymerase prior to the complex formation reaction; lanes 5 to 8, added after the complex formation reaction. In lanes 5 and 6, ATP and CTP were added after rifampin and incubated for an additional 10 min.

## RESULTS

**Rifampin inhibits strong melting during open complex formation in vitro.** To compare with in vivo results of rifampin treatment, we first assayed the in vitro effects of the drug on the *rrnB* $P_1$ promoter. Previous studies on this promoter have shown that both of the two initiating nucleotides ATP and CTP are required to form heparin-stable open complexes (9), and the performed pppApC dinucleotide does not replace the requirement for ATP and CTP. From these observations, one might predict that rifampin would inhibit the formation of these complexes because of its inhibitory effect on the formation of the first dinucleotide bond.

Figure 1 shows that rifampin does indeed strongly inhibit the formation of these melted complexes. The $KMnO_4$-hyperreactive sites indicated by the labeled arrow in lane 1 indicates the level of open complexes formed on $P_1$ in the presence of ATP and CTP (compare with the naked DNA control in lane 9). When rifampin is preincubated with the RNA polymerase prior to DNA addition (lane 3), the signal is severely diminished but not abolished. Densitometric scanning of the autoradiograph shows that a 10-fold decrease in open complex levels has accompanied the rifampin treatment. A similar decrease occurs when rifampin is added after a 10-min preincubation of polymerase with DNA (lane 5). Thus, rifampin inhibits strong melting at *rrnB* $P_1$ significantly. This is a unique property, as it contrasts with the general effect of rifampin on *E. coli* promoters (29).

The unusual rifampin sensitivity is likely related to the special requirement of *rrnB* $P_1$ for the initiating nucleotides ATP and CTP. When the polymerase and DNA are incubated with ATP and CTP prior to the addition of rifampin, strong melting occurs (Fig. 1, lane 7). Thus, the addition of the initiating nucleotides protects against the ability of rifampin to block strong melting. This may simply be because nucleotides are required for strong, stable melting and rifampin inhibition is ineffective after the initiating nucleotides are used.

The ability of strongly melted open complexes to elongate was assayed. When UTP and GTP are added to a complex formed in the presence of ATP and CTP, transcriptional elongation occurs, as evidenced by a marked decrease in the level of open complex signal (Fig. 1; compare lane 2 with lane 1). This control demonstrates that the strongly melted open complexes are functional. If rifampin is added to these complexes prior to addition of UTP and GTP, the complexes retain their ability to elongate (reduced signal in lane 8). Thus, the initiated open complexes are both functional and rifampin resistant, as expected. However, if rifampin is added along with ATP and CTP, the complexes containing a low permanganate signal cannot elongate, as demonstrated by the lack of a decrease in signal when the remaining two nucleotides are added (lane 3 versus lane 4 and lane 5 versus

lane 6). We show elsewhere (22) that polymerase in vitro is strongly occupying the promoter whether or not initiating nucleotides are present.

These results show that the strongly melted open complex has properties expected of complexes that have progressed past the typical open complex stage and have initiated transcription. This complex is not formed in the presence of rifampin. Once it has formed, rifampin cannot block its ability to elongate; melting is reduced upon elongation, since the start site recloses and strong remelting is blocked by the rifampin. These properties resemble those of the initiated complexes (5) and initial transcribing complexes (12) detected at other promoters, rather than typical open complexes. To avoid confusion, we will refer to this complex as an open complex, with the understanding that the nucleotide requirement causes it to have unusual properties.

**Rifampin does not alter melting in vivo.** As mentioned above, probing of *rrnB* $P_1$ on the chromosome is potentially complicated by the existence of seven copies of rRNA-encoding operons, all of which are quite similar. All seven have both a $P_1$ and a $P_2$ promoter, which are spaced at a conserved distance of approximately 120 bp, and all drive the transcription of the 16S-23S-5S rRNA polycistronic message. Starting at approximately the 30th nucleotide downstream of the $P_2$ start site, all of the chromosomal copies of the rRNA operons have the same DNA sequence, up to and including the coding region of the 16S rRNA. Thus, one cannot design an oligonucleotide primer that hybridizes uniquely to the *rrnB* region of the chromosome from the downstream position. However, the sequences in the regions upstream of these operons diverge, and unique sequences are present upstream of nucleotide −50. Thus, a unique primer which hybridizes the region upstream of the $P_1$ promoter and reads downstream can be designed.

Such a primer was synthesized and was confirmed to hybridize specifically to *rrnB* by comparing the dimethyl sulfate attack pattern in vivo and in vitro with the sequence of an *rrnB*-containing plasmid as read by the same primer (not shown). We also show below that the $P_1$ and $P_2$ permanganate-sensitive melting is in the same position in vitro and in vivo. This same primer was used in the preceding in vitro experiments to probe the *rrnB*-containing plasmid.

The in vivo probing with permanganate was similar to experiments at other promoters (27, 28). To obtain a snapshot of the state of the chromosome in vivo, growing cells were treated with $KMnO_4$, and the chromosomal DNA was rapidly isolated. The DNA was denatured and hybridized with the primer, and subsequently primer extension was used to locate the permanganate-hypersensitive sites. The result shows a prominent $P_1$ hypersensitive site (Fig. 2, lane 1) mapping to the same position as observed in the in vitro systems described above. This is easily detectable even in the absence of the inhibitor rifampin.

Previous studies at *lac* and *glnAp*$_2$ have shown large signal increases upon rifampin addition in vivo (27, 28). However, addition of the drug rifampin in vivo does not alter the melting signal for the $P_1$ promoter (Fig. 2, lane 2). As an internal control for this unusual result, the $P_2$ melting signal in the same samples is enhanced by the addition of rifampin (lane 2 versus lane 1 and lane 4 versus lane 3).

Next, bacteria were probed under conditions of amino acid starvation, which are known to be accompanied by large increases in the intracellular concentrations of ppGpp (6, 15) and result in selective inhibition of stable RNA synthesis (16). Isoleucine starvation was induced by the addition of high concentrations of valine to the media, which results in the feedback inhibition of the isoleucine and valine biosynthetic enzymes (24), ultimately starving the cells for isoleucine and increasing ppGpp levels dramatically (6). This treatment has been shown to induce the synthesis of large amounts of ppGpp (6) and result in selective inhibition of stable RNA synthesis (16).

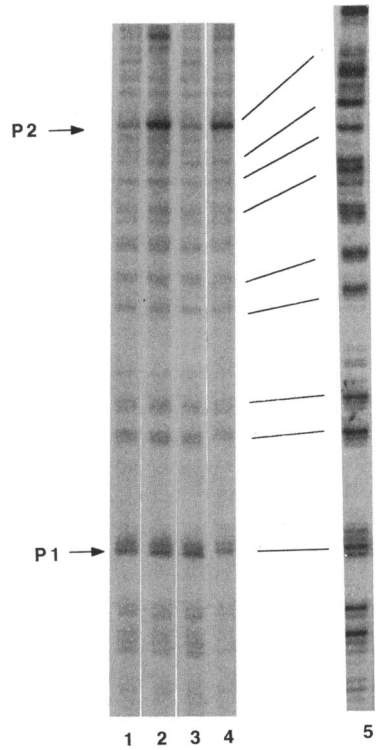


FIG. 2. In vivo footprinting of *rrnB* on the chromosome, determined by $KMnO_4$ footprinting of growing (lanes 1 and 2) and starving (lanes 3 and 4) cells. Rifampin was added to the cultures 5 min prior to harvesting in lanes 2 and 4. Lane 5 shows the $KMnO_4$-induced pattern of naked DNA.

Permanganate was added to the cells, confirmed to be starving by following growth, and the result of primer extension analysis is shown in Fig. 2, lane 3. The typical melting signal is still present and has not changed in intensity (compare lane 3 with lanes 1 and 2). The addition of rifampin also does not increase the signal under these conditions (compare lane 4 with lane 3).

We infer from these results that under all conditions, the observed permanganate signal is due to polymerase in a state that is not altered by rifampin. Since strongly melted open complexes in vitro can elongate but cannot re-form in the presence of rifampin (Fig. 1), such complexes would be expected to disappear upon rifampin treatment. Thus, the observed permanganate signal in vivo is much more likely to be analogous to the weak signal associated with polymerase that has not yet used ATP and CTP to form a stable, strongly melted complex.

**Rifampin does not alter melting during steady-state transcription in vitro.** In an attempt to assemble an in vitro system that is roughly comparable to the chromosomal situation, we mimicked ongoing steady-state transcription conditions, such as are presumed to exist in vivo. These conditions included high concentrations of nucleotides as

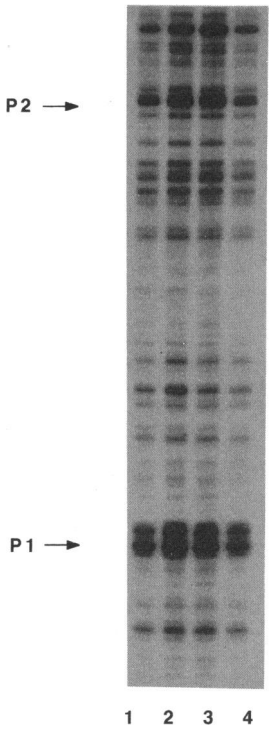


FIG. 3. Complex levels during steady-state transcription, determined by $KMnO_4$ footprinting during steady-state transcription (see Materials and Methods) in the absence (lanes 1 and 3) and presence (lanes 2 and 4) of rifampin and in the absence (lanes 1 and 2) and presence (lanes 3 and 4) of ppGpp. Hypersensitive sites corresponding to the −10 consensus regions of the two promoters are designated by labeled arrows.

well as high concentrations of RNA polymerase, which should promote free initiation and elongation on the highly transcriptionally competent supercoiled *rrnB* template, at least for a short time.

When $KMnO_4$ is added to the steady-state transcription system, the pattern shown in Fig. 3, lane 1, is observed. A permanganate-hypersensitive site is seen at the usual position. When rifampin is added to the ongoing steady-state transcription system (lane 2), no significant change in the $P_1$ signal is seen when the band intensity is normalized to that of background bands. This result contrasts sharply with the large decrease in signal induced by rifampin under non-steady-state conditions (Fig. 1, lane 3 versus lane 1). The signal also is not significantly altered by the addition of ppGpp (lane 3 versus lane 4). Thus, neither rifampin nor ppGpp changes the level of melting significantly in this purified system.

This result of steady-state studies in vitro is identical to the result of steady-state studies in vivo (Fig. 2). In both cases, the polymerase exists in a state wherein addition of rifampin does not lead to a change in permanganate signal. As discussed above, this is a property of polymerases existing prior to the use of ATP and CTP to assist in forming a strongly melted open complex.

## DISCUSSION

These experiments have shown that during steady-state transcription in vivo, the permanganate sensitivity of the *rrnB* $P_1$ promoter is not altered by addition of rifampin or elevation of ppGpp concentrations. These properties were mimicked in a steady-state transcription system in vitro. Other in vitro studies showed that polymerases that had used ATP and CTP to form strongly melted open complexes do not have these properties. Instead, polymerases that have used ATP and CTP can elongate but cannot remelt the DNA strongly in the presence of rifampin. This leads to a rifampin-induced lessening of melting, which is not observed in the in vivo experiments. Thus, the weak and constant permanganate sensitivity observed in vivo appears to be due to polymerases that have not yet used ATP and CTP to form strongly melted open complexes.

Polymerases in this state appear to predominate during steady-state transcription in vivo and in vitro despite the presence of high concentrations of ATP and CTP. We show elsewhere (22) that polymerase in vitro is strongly occupying the promoter, even when ATP and CTP are absent. Thus, the ATP- and CTP-dependent strong melting of the promoter appears to be the rate-limiting step in the transcription pathway (see also reference 14). This leads to an accumulation of complexes that slowly use ATP and CTP to form strongly melted open complexes (model in Fig. 4). We cannot currently distinguish whether these nucleotides are used to drive the opening of the DNA (21) or instead to stabilize transiently opened DNA strands (17). As discussed elsewhere (21, 22), the unusual difficulty in accumulating stable open complexes may be a consequence of the unusual *rrnB* $P_1$ promoter sequence. This promoter and others under stringent control have very G+C-rich melted regions (13) that may be difficult to open. The complexes that exist prior to this strong melting are predominantly closed but do show weak permanganate sensitivity at the same position as in open complexes (19, 21, 22).

If polymerase is rate limited at this step, how could ppGpp regulation work? One possibility is that ppGpp simply further slows this rate-limiting step by binding the polymerase and slowing the ATP- and CTP-dependent accumulation of strongly melted open complexes. This process would not lead to accumulation of more polymerase at the promoter, since polymerase would already have collected there, but would slow the rate at which polymerases proceed along the initiation pathway. In principle, any promoter that was difficult to melt might have this property. The model is consistent with the observation that the G+C-rich discriminator region is a critical determinant of ppGpp sensitivity (13).

The Fis protein has been shown to interact with the

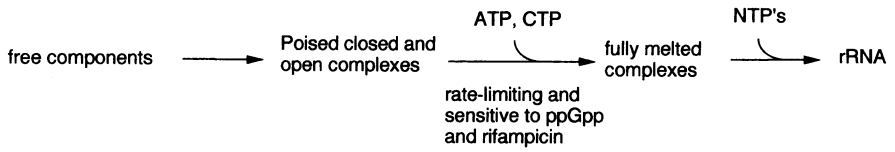


FIG. 4. Postulated model of the transcription cycle for *rrnB*. NTP's, nucleoside triphosphates.

upstream activation region (UAS) region of *rrnB* and increase transcription in vitro under certain solution conditions, including low concentrations of polymerase (25). Lack of the UAS can reduce transcription in vivo, especially under conditions of initial outgrowth from the stationary state (20). One potential mechanism for this activity is that the bound Fis protein attracts RNA polymerase to the promoter under conditions in which polymerase is limiting. The Fis-UAS interaction is without consequence for the stringent control mediated by ppGpp (25). Thus, Fis might increase promoter occupancy under certain conditions not tested here, but the ppGpp regulation of melting may not be changed by Fis.

The investigations presented here have addressed only two extreme growth conditions, fully supplemented growth and starvation. However, rRNA promoters have been shown to be subject to growth control as well and thus are differentially regulated by intermediate growth conditions. These studies cannot contribute to the resolution of the controversy as to the extent of growth control regulation by ppGpp (1, 8); nonetheless, the prediction from these studies is that polymerases should be present on the promoters, poised to form stable open complexes, under all intermediate growth conditions, since they are present under the extreme conditions tested here.

## ACKNOWLEDGMENTS

We thank Richard Gourse for his interest and forthright communications.

This research was supported by Public Health Service grant GM35754. K.L.O. was a trainee under Public Health Service National Research Service Award GM07185.

## REFERENCES

1. **Baracchini, E., and H. Bremer.** 1988. Stringent and growth control of rRNA synthesis in Escherichia coli are both mediated by ppGpp. J. Biol. Chem. **263**:2597–2602.
2. **Brosius, J., T. J. Dull, D. D. Sleeter, and H. F. Noller.** 1981. Gene organization and primary structure of a ribosomal RNA operon from Escherichia coli. J. Mol. Biol. **148**:107–127.
3. **Burgess, R. R., and J. J. Jendrisak.** 1975. A procedure for the rapid, large-scale purification of Escherichia coli DNA-dependent RNA polymerase involving polymin P precipitation and DNA-cellulose chromatography. Biochemistry **14**:4634–4638.
4. **Carpousis, A. J., and J. D. Gralla.** 1980. Cycling of ribonucleic acid polymerase to produce oligonucleotides during initiation in vitro at the lac UV5 promoter. Biochemistry **19**:3245–3253.
5. **Carpousis, A. J., and J. D. Gralla.** 1985. Interaction of RNA polymerase with lac UV5 promoter DNA during mRNA initiation and elongation: footprinting, methylation, and rifampicin-sensitivity changes accompanying transcription initiation. J. Mol. Biol. **183**:165–177.
6. **Cashel, M., and J. Gallant.** 1969. Two compounds implicated in the function of the RC gene of Escherichia coli. Nature (London) **221**:838–841.
7. **Cashel, M., and K. Rudd.** 1987. The stringent response, p. 1410–1438. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology, vol. 2. American Society for Microbiology, Washington, D.C.
8. **Gaal, T. R., and R. L. Gourse.** 1990. Guanosine 3′-diphosphate 5′-diphosphate is not required for growth rate-dependent control of rRNA synthesis in Escherichia coli. Proc. Natl. Acad. Sci. USA **87**:5533–5537.
9. **Gourse, R. L.** 1988. Visualization and quantitative analysis of complex formation between E. coli RNA polymerase and an RNA promoter in vitro. Nucleic Acids Res. **16**:9789–9809.
10. **Gralla, J. D.** 1990. Promoter recognition and mRNA initiation by *E. coli* $E\sigma^{70}$. Methods Enzymol. **185**:37–54.
11. **Johnston, D. E., and W. R. McClure.** 1976. Abortive initiation of in vitro RNA synthesis on bacteriophage lambda DNA, p. 413–428. *In* R. Losick and M. Chamberlin (ed.), RNA polymerase. Cold Spring Harbor Monograph Series. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
12. **Krummel, B., and M. J. Chamberlin.** 1989. RNA chain initiation by Escherichia coli RNA polymerase: structural transitions of the enzyme in early ternary complexes. Biochemistry **28**:7829–7842.
13. **Lamond, A. I., and A. A. Travers.** 1985. Stringent control of bacterial transcription. Cell **41**:6–8.
14. **Langert, W., M. Meuthen, and K. Mueller.** 1991. Functional characteristics of the rrnD promoters of Escherichia coli. J. Biol. Chem. **266**:21608–21615.
15. **Lazzarini, R. A., M. Cashel, and J. Gallant.** 1971. On the regulation of guanosine tetraphosphate levels in stringent and relaxed strains of Escherichia coli. J. Biol. Chem. **246**:4381–4385.
16. **Lazzarini, R. A., and A. F. Dahlberg.** 1971. The control of ribonucleic acid synthesis during amino acid deprivation in Escherichia coli. J. Biol. Chem. **264**:420–429.
17. **Leirmo, S., and R. L. Gourse.** 1991. Factor-independent activation of Escherichia coli rRNA transcription. I. Kinetic analysis of the roles of the upstream activator region and supercoiling on transcription of the *rrnB* $P_1$ promoter in vitro. J. Mol. Biol. **220**:555–568.
18. **McClure, W. R.** 1985. Mechanism and control of transcription initiation in prokaryotes. Annu. Rev. Biochem. **54**:171–204.
19. **Newlands, J. T., W. Ross, K. K. Gosink, and R. L. Gourse.** 1991. Factor-independent activation of Escherichia coli rRNA transcription. II. Characterization of complexes of rrnB P1 promoters containing or lacking the upstream activator region with Escherichia coli RNA polymerase. J. Mol. Biol. **220**:569–583.
20. **Nilsson, L., A. Vanet, E. Vijgenboom, and L. Bosch.** 1990. The role of Fis in trans activation of stable RNA operons of E. coli. EMBO J. **9**:727–734.
21. **Ohlsen, K. L., and J. D. Gralla.** 1992. Interrelated effects of DNA supercoiling, ppGpp, and low salt on melting within the *E. coli* ribosomal RNA *rrnB* $P_1$ promoter. Mol. Microbiol., in press.
22. **Ohlsen, K. L., and J. D. Gralla.** 1992. DNA melting within stable closed complexes at the *E. coli rrnB* $P_1$ promoter. J. Biol. Chem., in press.
23. **Owen, R. J., and P. Borman.** 1987. A rapid biochemical method for purifying high molecular weight bacterial chromosomal DNA for restriction analysis. Nucleic Acids Res. **15**:3631.
24. **Ramakrishnan, T., and E. A. Edelman.** 1965. Regulatory mechanisms in the biosynthesis of isoleucine and valine. II. Identification of two operator genes. J. Bacteriol. **89**:654–660.
25. **Ross, W., J. F. Thompson, J. T. Newlands, and R. Gourse.** 1990. E. coli Fis protein activates ribosomal RNA transcription in vitro and in vivo. EMBO J. **9**:3733–3742.
26. **Sasse-Dwight, S., and J. D. Gralla.** 1988. Probing co-operative DNA-binding in vivo: the lac $O_1$:$O_3$ interaction. J. Mol. Biol. **202**:107–119.
27. **Sasse-Dwight, S., and J. D. Gralla.** 1988. Probing the Escherichia coli glnALG upstream activation mechanism in vivo. Proc. Natl. Acad. Sci. USA **85**:8934–8938.
28. **Sasse-Dwight, S., and J. D. Gralla.** 1989. $KMnO_4$ as a probe for lac promoter DNA melting and mechanism in vivo. J. Biol. Chem. **264**:8074–8081.
29. **Sippel, A., and G. Hartmann.** 1968. The mode of action of rifamycin on the RNA polymerase reaction. Biochim. Biophys. Acta **157**:218–219.