Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 2908–2913, March 1998
Biochemistry

The *rpoB* mutants destabilizing initiation complexes at stringently controlled promoters behave like "stringent" RNA polymerases in *Escherichia coli*

YAN NING ZHOU AND DING JUN JIN*

Laboratory of Molecular Biology, Building 37, Room 2E14, National Cancer Institute, National Institutes of Health, 9000 Rockville Pike, Bethesda, MD 20892

Communicated by Carol A. Gross, University of California, San Francisco, CA, January 6, 1998 (received for review July 18, 1997)

ABSTRACT **In *Escherichia coli*, stringently controlled genes are highly transcribed during rapid growth, but "turned off" under nutrient limiting conditions, a process called the stringent response. To understand how transcriptional initiation at these promoters is coordinately regulated, we analyzed the interactions between RNA polymerase (RNAP) (both wild type and mutants) and four stringently controlled promoters. Our results show that the interactions between RNAP and stringently controlled promoters are intrinsically unstable and can alternate between relatively stable and metastable states. The mutant RNAPs appear to specifically further weaken interactions with these promoters in vitro and behave like "stringent" RNAPs in the absence of the stringent response *in vivo*, constituting a novel class of mutant RNAPs. Consistently, these mutant RNAPs also activate the expression of other genes that normally require the response. We propose that the stability of initiation complexes is coupled to the transcription of stringently controlled promoters, and this unique feature coordinates the expression of genes positively and negatively regulated by the stringent response.**

Under optimal growth conditions, rapidly dividing *Escherichia coli* cells transcribe a set of genes at a very high rate. These genes engage most of the RNA polymerase (RNAP) molecules in the cell, although they constitute only a small fraction of the genome. In contrast, nutrition-limiting conditions, such as amino acid starvation, cause a rapid accumulation of the guanine derivative, ppGpp, and a dramatic reduction in expression of these genes, a process termed the stringent response (1). Because expression of these genes is negatively regulated during the stringent response in a manner dependent on the allelic state of the *relA* gene (2), they are called stringently controlled (or stringent) genes.

Most of the stringent genes encode translational machinery, but some encode mRNAs. One such example is *pyrBI*, a pyrimidine biosynthetic operon, whose expression is inhibited during the stringent response (3). Thus, the expression of operons encoding rRNA, such as *rrnB P1*, and those encoding nucleotide biosynthetic enzymes, such as *pyrBI*, are coordinately regulated during the stringent response, even through each of these operons also has its own unique regulatory feature(s) (4–6). The stringent response serves as a global regulatory mechanism, coordinating the transcriptional activity of RNAP with the growth conditions of the cell.

Despite the biological importance of the stringent response and extensive studies in the past decades, the mechanism(s) by which stringently controlled genes are coordinately regulated has been elusive (1). To further understand the regulation of stringently controlled genes, we decided to analyze RNAP mutants that appeared to have specifically altered interaction with stringently controlled (or stringent) promoters. Studying such RNAP mutants and defining the nature of their defects in interaction with this class of promoters might help us to understand the mechanism of the stringent response in the cell.

Recently, we found that four *rpoB* mutations [*rpoB114*(S531F), *rpoB3449*(Δ532A), *rpoB3443*(L533P), and *rpoB3370*(T563P)] (7), which are among previously characterized Rif^r alleles (8, 9), have specifically reduced the transcription from the two major promoters, *P1* and *P2*, of the *rpoD* operon (10, 11), leading to a hyper-temperature-sensitive phenotype of a σ^{70} mutant (9). Because these two promoters are negatively controlled by the stringent response (12), it suggests that these *rpoB* mutants have specifically altered interaction with stringent promoters.

Interestingly, these four *rpoB* mutations also increase expression of some genes positively regulated by the stringent response. In the *relA spoT* double-deletion strains lacking ppGpp, cells could not grow in minimal media without a supplement of amino acids, because several amino acid biosynthetic operons are positively controlled by the stringent response (13). We found that among the Rif^r mutations previously characterized (8), the same four described here enabled the *relA spoT* double-deletion mutants to grow in minimal media (H. Murphy, D.J.J., and M. Cashel, unpublished results; also see ref. 1). Thus, these RNAP mutants behave like "stringent" RNAPs in the absence of the stringent response *in vivo*.

In this study, we asked the following two questions: (*i*) Are these RNAP mutants also defective in transcription of other stringent promoters? If they are, it would indicate that the RNAP mutants have an altered response to a unique feature common to this class of promoters. (*ii*) Is there a common mechanism underlying the defects of these RNAP mutants in the interaction with stringent promoters? To address these questions, we analyzed the interactions between RNAP (both wild type and the mutants) and four stringent promoters (*rrnB P1*, *pyrBI*, and *P1* and *P2* of the *rpoD* operon). We have identified a unique feature common to this class of promoters and propose a model mechanism explaining the coordinated regulation of the stringent response.

MATERIALS AND METHODS

Bacteria Strains and Bacterial Techniques. The *E. coli* strains are K12 MG1655 derivatives. The *rpoB* mutations were described and introduced into different strain backgrounds as described (8). All λ monolysogens carrying promoter-*trp–lacZ* fusions were from Wilma Ross and Rick Gourse (University of Wisconsin-Madison): RLG1350 is MG1655 Δ*lacX74*/λ*rrnB P1* (−81–+1)–*lacZ* and RLG1351 is the *fis*::*kan* derivative of RLG1350 (5); RLG1319 is MG1655 Δ*lacX74*/λ*lacUV5–lacZ* (4), and RLG1320 is the *fis*::*kan* derivative of RLG1319. The basic bacterial techniques were as described (7, 14). β-Galactosidase activities were determined by using log-phase cultures grown in Luria–Bertani (LB) medium at 30°C by two methods: one was as described (14), and the other was

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

0027-8424/98/952908-6$0.00/0
PNAS is available online at http://www.pnas.org.

Abbreviations: RNAP, RNA polymerase; IHF, integration host factor.

*To whom reprint requests should be addressed. e-mail: djjin@helix.nih.gov.

by using SPECTRAmax 250 (Molecular Devices) according to the manufacturer's manual. The levels of integration host factor (IHF) were determined by using log-phase cultures grown in LB medium at 30°C by anti-IHF antibody after the total protein extracts were prepared and separated with a 16% tricine gel as described (7).

Chemicals and Reagents. Nucleotides were purchased from Boehringer Mannheim, chemicals and poly[d(A·T)] were from Sigma, pKK223–3 was from Pharmacia Biotech, and ^{32}P-labeled nucleotides were from Amersham. Plasmid pRLG1617 [contains *rrnB P1* (−81–+1)–*rrnB* T1T2] (5) was from Wilma Ross and Richard Gourse, and pBHM332 [contains the *pyrBI* promoter-attenuator (758-bp *Pvu*II fragment)] was described (15); pDJ54 [contains the *P1* and *P2* promoters of the *rpoD* operon-*rpoC* T] was described (7). Supercoiled plasmid DNA of native superhelical density and DNA fragments were purified as described (15). RNAPs were purified from wild-type *E. coli* K12 MG1655 and different *rpoB* mutant strains as described (16).

***In Vitro* Transcription.** Reactions containing either KCl or K-glutamate salts were as described (7, 15). For transcription of *rrnB P1*, reactions containing ≈3 nM of supercoiled DNA (pRL1617) and 2–4 nM RNAP were performed at ≈23°C. For transcription of other promoters, reactions containing ≈5 nM DNA and 20 nM RNAP were performed at 37°C. RNAP and DNA templates were preincubated for ≥15 min, and reactions were started by the addition of NTPs (0.2 mM for ATP, GTP, and CTP, and 0.02 mM for UTP including about 5 μCi of [^{32}P]UTP). When indicated, heparin (100 μg/ml), poly[d(A·T)] (125 μg/ml), or pKK223–3 (≈40 nM) was added with NTPs to restrict transcription to a single round by binding to free RNAP molecules. After 15 min, reactions were stopped and analyzed on an 8% sequencing gel followed by autoradiography, as described (15).

Determination of the Kinetics of Inactivation of Initiation Complexes by Inhibitors. Conditions were the same as described above for *in vitro* transcription with the following modifications. After preincubation of RNAP and DNA template, either pKK223–3 plasmid or heparin was added into reactions at time zero. At times indicated after the addition of inhibitor, NTPs were added, and reactions were allowed to continue for another 15 min before being stopped and analyzed as described above. The data were quantified either with ImageQuant PhosphorImager (Molecular Dynamics) or with Eagle Eye II (Stratagene).

RESULTS

The *rpoB* Mutants Are Defective in Transcription from *rrnB P1 in Vivo*. We analyzed the effects of these four *rpoB* mutations on transcription from *rrnB P1* by measuring β-galactosidase activity in strains containing a chromosomal *rrnB P1–lacZ* gene fusion (Table 1). The fusion contains an intact *rrnB P1* including the UAR sequence and the Fis protein binding site (5). Compared with the *rpoB*$^+$ isogenic strain, all four *rpoB* mutants had decreased transcription from the *rrnB P1* promoter, ranging from about a 2- to 4-fold reduction (Table 1, column 2). However, the defects of these *rpoB* mutations on transcription are promoter-specific, because the four *rpoB* mutants showed a 2- to 3-fold increase in expression from a nonstringent control promoter *lacUV5* compared with the wild-type strain (Table 1, column 4).

Table 1. The *rpoB* mutations specifically reduced transcription of *rrnB P1*-lacZ

	rrnB P1-lacZ		*lacUV5-lacZ*	
Strains	*fis*$^+$	*fis*$^-$	*fis*$^+$	*fis*$^-$
Wild type	1.00	0.76	1.00	1.85
rpoB114	0.57	0.56	3.00	4.61
rpoB3449	0.23	0.24	2.39	3.48
rpoB3443	0.33	0.29	2.76	4.92
rpoB3370	0.41	0.40	1.88	3.27

Values are the β-galactosidase activities normalized to the wild-type strains (*rpoB*$^+$ *fis*$^+$) for each promoter fusion. Numbers are the average of two independent determinations as described in *Materials and Methods*. Each determination was performed with minimum of two repetitions. The values of the two determinations were in close agreement.

Because the Fis protein is involved in the transcription of *rrnB*, the effects of the *rpoB* mutations on transcription from the promoter in a *fis* mutant also were determined. In the *rpoB*$^+$ background, transcription of *rrnB P1* was slightly reduced in the *fis* mutant as reported before (5), although expression of the *lacUV5* promoter increased in the *fis*$^-$ strain (Table 1, compare columns 3 and 5). We found that the defects of these rpoB mutations on the transcription from *rrnB P1* were independent of the *fis* allele (Table 1, compare columns 2 and 3).

The Mutant RNAPs Are Defective in Interaction with *rrnB P1 in Vitro*. Initiation of the *rrnB P1* promoter by wild-type RNAP is very sensitive to salt and heparin on linear DNA (17), but not on supercoiled DNA (18). Initially, we studied the interactions of purified RNAPs (wild type and three mutants) and *rrnB P1* by determining the effects of different DNA competitors on transcription of *rrnB P1* on supercoiled DNA (Fig. 1). The transcription from the *RNAI* promoter of the plasmid origin served as a control for nonstringent promoter.

Only wild-type RNAP was able to make significant amounts of the *rrn B P1* transcript in the presence of these inhibitors. Transcription of *rrn B P1* by the three mutant RNAPs was totally inhibited in the presence of heparin (Fig. 1, compare lane 2 to lanes 6, 10, and 14), and was considerably reduced or eliminated in the presence of poly[dA·dT] or pKK223–3 (Fig. 1, compare lanes 3 and 4 to lanes 7 and 8, 11 and 12, and 15 and 16). The differential behavior of the RNAP mutants toward different inhibitors is correlated to the fact that heparin is known to "attack" weakly bound RNAP molecules at a promoter (19), whereas the other two inhibitors do not. Transcription of *RNAI* and *tac* from the challenging DNA pKK223–3 by the mutant RNAPs was better or comparable to the wild-type enzyme. Similarly, we also found that these mutant RNAPs were very sensitive to salt in transcription of *rrnB P1* on supercoiled DNA (data not shown). Because the initiation complexes of *rrnB P1* with the mutant RNAPs could not survive the heparin challenge even when heparin was added with NTPs at the same time, it suggests that their half-lives are < 1 sec. In contrast, the half-life of wild-type RNAP·*rrnB P1* promoter complexes is ≈ 2.5 min under similar conditions (18). These results indicate that the *rpoB* mutations make the RNAP·*rrnB P1* promoter complexes less stable even with a supercoiled DNA template.

To determine whether the stability of the initiation complex of *rrnB P1* with the mutant RNAPs also is reduced in the presence of another DNA competitor, we compared the half-lives of the complexes formed between wild-type RNAP and RpoB3449 (the least defective mutant) by following the reduction of the *rrnB P1*

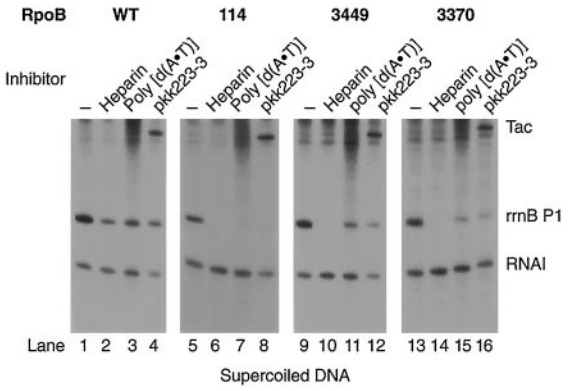

FIG. 1. *In vitro* transcription assays of *rrnB P1* with wild-type (WT) and mutant RNAPs. The experiment was performed either without (lanes 1, 5, 9 and 13) or with an indicated inhibitor as described in *Materials and Methods*. The transcripts from *rrnB P1* (≈170 nt), *RNAI* (≈110 nt), and *tac* are indicated.

transcript as a function of time in the presence of challenging plasmid DNA containing *Ptac* (Fig. 2). The half-life of the RNAP·*rrnB P1* promoter complex with wild-type RNAP was approximately 15 min, whereas, with RpoB3449, it was reduced to only about 4–5 min (Fig. 2*B*). The RNAP·*RNAI* promoter complexes were very stable with either the wild-type or mutant RNAP.

The Mutant RNAPs Are Also Defective in Interaction with the *pyrBI* Promoter. To determine whether the effects of the mutant RNAPs on *rrnB P1* transcription reflect a general defect in interaction with stringent promoters, another such promoter, *pyrBI*, was analyzed (Fig. 3). The synthesis of *pyrBI*, which is terminated at the natural attenuator present in the template, is sensitive to ppGpp, and thus, is subject to the stringent response (20). RNA synthesis from another promoter, *P*, located about 190-bp upstream of *pyrBI*, is not sensitive to ppGpp (20), and was used as a control.

We first determined the effects of the mutant RNAPs on transcription from *pyrBI* with a linear DNA template (Fig. 3*A*) under low UTP concentrations to avoid nonproductive initiation (6, 21). As expected, wild-type RNAP made a large amount of the transcript from *pyrBI* and a smaller amount from the upstream promoter *P* (20) (Fig. 3*A*, lane 1). In contrast, all three mutant RNAPs synthesized reduced amounts of RNA specifically from the *pyrBI* promoter (Fig. 3*A*, compare lane 1 to lanes 4, 7, and 10), and not from the *P* promoter. In the presence of heparin, transcription of *pyrBI* by wild-type RNAP was greatly reduced; whereas that by the mutant RNAPs was totally eliminated (Fig. 3*A*, compare lanes 2 and 3 to lanes 5 and 6, 8 and 9, and 11 and 12). None of the enzymes displayed sensitivity toward heparin in the transcription of promoter *P*.

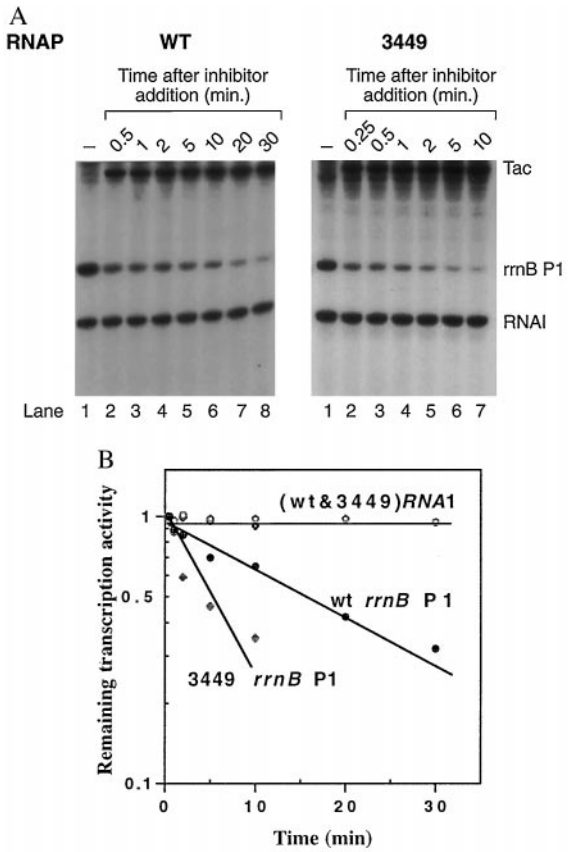

FIG. 2. Determining the stability of initiation complexes of *rrnB P1* with wild-type (WT) and the mutant RNAPs. The experiment was performed with 50 mM KCl in the presence of competitive DNA pKK223–3 (≈80 nM) as described in *Materials and Methods*. Note different time scales for different experiments. The controls without the inhibitor are indicated as −. (*A*) Autoradiogram of the transcripts. (*B*) The transcription activities from *rrnB P1* and *RNAI* were plotted as function of time (after inhibitor addition).

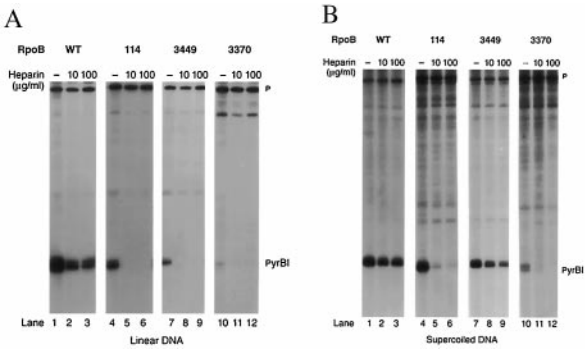

FIG. 3. *In vitro* transcription assays of *pyrBI* with wild-type (WT) and mutant RNAPs. The experiment was performed with 50 mM KCl as described in *Materials and Methods*. Heparin concentration is indicated when it was present. The transcripts from *pyrBI* (≈135 nt) and *P* (≈325 nt) are indicated. (*A*) Transcription was performed with linear DNA, *Pvu*II 758-bp fragment. (*B*) Transcription was performed with supercoiled DNA pBHM332.

With supercoiled DNA (Fig. 3*B*), the defects of the mutant RNAPs in transcription of *pyrBI* were suppressed to different degrees in the absence of heparin (compare Fig. 3 *A* to *B*). However, in the presence of heparin, the mutant RNAPs all were sensitive to the inhibitor, whereas wild-type RNAP became heparin-resistant. Even for the best-suppressed mutant, RpoB3449, the synthesis of *pyrBI* RNA was sensitive to the concentration of heparin, whereas wild-type RNAP was equally resistant to 10 and 100 μg/ml of the inhibitor (Fig. 3*B*, compare lanes 2 and 3 to lanes 8 and 9). Under all conditions, mutant RNAPs made either similar amounts or more of the *P* transcript compared with wild-type RNAP.

Supercoiled DNA Activates Transcription from the *pyrBI* Promoter. The effect of supercoiled DNA on transcription of *pyrBI* has not been reported. Our results showed that supercoiled DNA had a positive effect on the transcription of *pyrBI* in two respects: (*i*) it made transcription with wild-type RNAP heparin-resistant, and (*ii*) it partially suppressed transcription defects of the mutant RNAPs (compare Fig. 3 *A* to *B*), indicating that these two events are related. These properties suggest that the RNAP·*pyrBI* promoter complexes are stabilized by supercoiling, just like RNAP·*rrnB P1* promoter complexes. To test this, kinetics of transcription inactivation by heparin challenge of the RNAP·*pyrBI* complexes were determined.

With linear DNA, although the *pyrBI* promoter was very active in the absence of heparin, the RNAP·*pyrBI* promoter complexes were extremely unstable, with a half-life of seconds, as no *pyrBI* transcript could be detected immediately after the addition of heparin (Fig. 4*A*). However, transcription from the promoter *P* was very resistant to the heparin challenge. As predicted, with supercoiled DNA, the RNAP·*pyrBI* promoter complexes were stabilized with a half-life of about 40 min (Fig. 4*B*). However, they were still less stable than the initiation complexes of the control promoter *P*.

The suppression of the defects of the mutant RNAPs in transcription of *pyrBI* on supercoiled DNA was not complete (Fig. 3*B*), suggesting that these mutant RNAP·*pyrBI* promoter complexes are less stable than the wild type. Consistently, in a heparin challenge experiment with the least-defective RNAP, RpoB3449, the stability of the mutant RNAP·*pyrBI* promoter complexes was greatly reduced, with a half-life less than 0.5 min (Fig. 4*C*). However, the initiation complexes of promoter *P* were very stable under the same conditions, comparable to those of wild-type RNAP.

The Nature of the Defect of the RNAP Mutants in Interaction with the Two Major Promoters of the *rpoD* Operon Is the Same as Those with *rrnB P1* and *pyrBI*. Although the two major

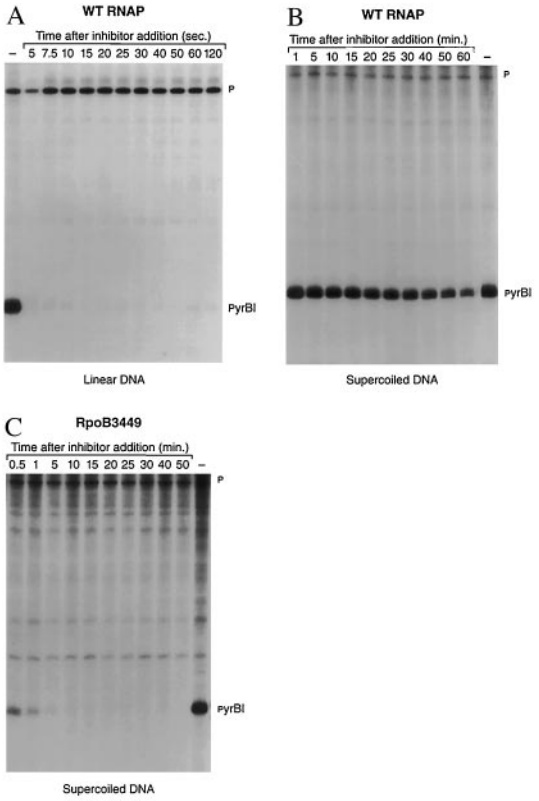

FIG. 4. Determining the stability of RNAP·*pyrBI* complexes under different conditions. Experiment was performed with 100 mM K-glutamate in the presence of heparin (10 μg/ml) as described in *Materials and Methods*. Note different time scales for different experiments. The controls without the inhibitor are indicated as −. (*A*) Wild-type (WT) RNAP on linear DNA (*Pvu*II 758-bp fragment). (*B*) WT RNAP on supercoiled DNA pBHM332. (*C*) RpoB3449 on supercoiled DNA pBHM332.

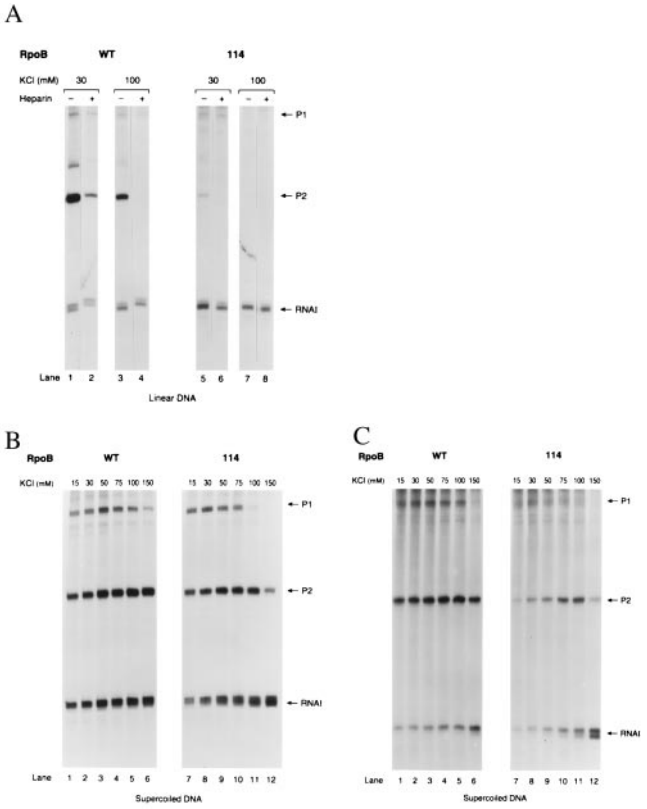

FIG. 5. *In vitro* transcription assays of *P1* and *P2* of the *rpoD* operon with wild-type (WT) RNAP and RpoB114. Transcription was performed as described in *Materials and Methods*. The transcripts form *P1* (≈160 nt) and *P2* (≈240 nt) of the *rpoD* operon and RNAI (≈110 nt) are indicated. (*A*) Transcription was performed on linear DNA (pDJ54 DNA that was digested with *Bam*HI) with different concentrations of KCl (either with or without heparin). (*B*) Transcription was performed on supercoiled DNA pDJ54 with different concentrations of KCl in the absence of heparin. (*C*) Same as in *B* except that heparin was present.

promoters, *P1* and *P2*, of the *rpoD* operon were shown to be stringently controlled *in vivo* (12), their *in vitro* behavior has not been studied. Nor has the nature of the defect of these RNAP mutants in interaction with these two stringent promoters been analyzed (7). Based on the similarities of the behavior of wild-type RNAP with *rrnB P1* and *pyrBI*, we predicted that the *P1* and *P2* promoters of the *rpoD* operon also would be similar to *rrnB P1* and *pyrBI*. Furthermore, based on the similar behavior of these RNAP mutants with *rrnB P1* and *pyrBI*, we predicted that the defects of these RNAP mutants in interaction with the *P1* and *P2* promoters of the *rpoD* operon also would be similar to those with *rrnB P1* and *pyrBI*. The experiments described below support these two predictions. In these *in vitro* transcription assays (Fig. 5), we analyzed the transcription of wild-type RNAP and RpoB114, a representative of the RNAP mutants, with the DNA template containing the *P1* and *P2* promoters of the *rpoD* operon and a very strong rho-independent terminator of *rpoC* downstream from the promoters (7).

With a linear DNA template (Fig. 5*A*), for wild-type RNAP, the transcription of *P1* and *P2* of the *rpoD* operon was very sensitive to both salt concentration and inhibitor heparin when compared with that of RNAI, a control nonstringent promoter. The mutant RNAP was essentially inactive in synthesizing the *P1* and *P2* transcripts. However, the transcription of RNAI by RpoB114 was very active and resistant to salt and heparin.

With supercoiled DNA template in the absence of heparin (Fig. 5*B*), wild-type RNAP was able to synthesize significant amounts of *P1* transcript and large amounts of *P2* transcript in KCl concentrations ranging from 15 to 150 mM, although the synthesis of *P1* was still sensitive to the KCl concentration. This finding indicates that supercoiled DNA activates the transcription at the two promoters. Similarly, supercoiled DNA largely corrected the defect of the mutant RNAP in transcription of *P1* and *P2*, but RpoB114 was still more salt-sensitive than the wild-type RNAP.

With supercoiled DNA template in the presence of heparin (Fig, 5*C*), wild-type RNAP was still able to synthesize *P1* and *P2*, except that the production of *P2* was reduced at 150 mM. However, compared with wild-type RNAP, the synthesis of *P1* and *P2* by RpoB114 was greatly reduced, as judged by the ratios of the activities of these two promoters to that of the control promoter RNAI. Interestingly, the transcription of RNAI by both wild-type and the mutant RNAP was increased as the KCl concentration increased regardless of whether heparin was present (Fig. 5 *B* and *C*).

For wild-type RNAP, the reduced transcription activity from *P1* and *P2* with linear DNA was correlated with the extreme instability of the RNAP·promoter complexes (Fig. 6). With linear DNA, *P2* was very active but the activity of *P1* was minimal in the absence of heparin (Fig. 6, lane 1). The half-life of the RNAP·*P2* promoter complexes with linear DNA was only about 25 sec. With a supercoiled DNA template, the complexes of RNAP and the two promoters were much more stable, with a half-life of about 35 min for *P1* and 25 min for *P2*. These results confirm that supercoiled DNA stabilizes these complexes. However, they were still less stable than the initiation complexes from the control promoter RNAI.

For RpoB114, its defect in transcription of *P1* and *P2* of the *rpoD* operon also was correlated with its increased instability of the complexes between the mutant RNAP and the promoters specifically (Fig. 6). With supercoiled DNA template, we found that the stability of the complexes of the mutant RNAP and the two

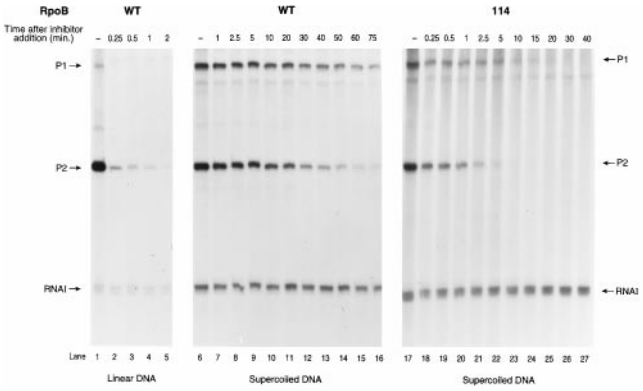

FIG. 6. Determining the stability of the complexes formed between RNAP and the *P1* and *P2* promoters of the *rpoD* operon. Experiments were performed with 50 mM KCl in the presence of heparin (100 μg/ml) as described in *Materials and Methods*. Note different time scales for different experiments. The controls without the inhibitor are indicated as −. Transcriptions were performed with wild-type (WT) RNAP on linear DNA (pDJ54 DNA that was digested with *Bam*HI), and with WT RNAP and RpoB114 on supercoiled DNA pDJ54 as indicated.

promoters were greatly reduced when compared with wild-type RNAP, with a half-life of 5 min and 1.5 min for *P1* and *P2*, respectively. The RpoB114·RNAI promoter complexes were very stable, just like that of wild-type RNAP.

DISCUSSION

In this study, we analyzed the interactions of RNAP (wild type and four RNAP mutants that showed "stringent" phenotypes in the absence of the stringent response *in vivo*) and four stringent promoters (*rrnB P1*, *pyrBI*, *P1* and *P2* of the *rpoD* operon). We made two important findings. (*i*) The behavior of these four promoters is very similar: the interactions of RNAP and these stringent promoters are intrinsically unstable and are greatly influenced by the superhelicity of DNA templates. Such a feature previously was described only for *rrnB P1*, but our results generalize it to three other stringent promoters. It is very likely that this will prove to be a common feature for stringent promoters in general. (*ii*) The behavior of these RNAP mutants toward these four stringent promoters is very similar: the RNAP mutants further destabilize the interactions with this class of promoters. Based on these two findings, we propose that the instability of the RNAP·stringent promoter complexes is the key element regulating this class of promoters during the stringent response.

A Unique Feature of the Interaction between RNAP and Stringent Promoters. Our study clearly demonstrated that there is a common feature shared by the four stringent promoters used in this work. These promoters are all hypersensitive to salt, heparin, and superhelicity of DNA template. The complexes formed between RNAP and stringent promoters on linear DNA template are extremely unstable and their half-lives last only seconds, the least-stable complexes in *E. coli* known to us at the present time. [For example, the half-life of initiation complexes of a nonstringent strong promoter, T7A1, is about 30 min on linear DNA template (22), a value regarded as relatively unstable compared with other nonstringent promoters in *E. coli*.] However, the half-lives of the complexes formed between RNAP and stringent promoters on supercoiled DNA template are relatively stable and resistant to salt and heparin. Although we have not systematically studied all other stringent promoters, our results strongly indicate that this unique feature is a hallmark of this class of promoters.

Part of the rif-Region of RNAP Is Important in Interaction with Stringent Promoters. Our results show that the mutant RNAPs amplify the intrinsic instability of the interactions between RNAP and stringent promoters. In effect, their interaction with stringent promoters on supercoiled DNA mimics that of wild-type RNAP on linear DNA. Overall, the nature of the defects of these mutant RNAPs interacting with these four stringent promoters is comparable, although the degree of the defect of each mutant RNAP at each promoter is not necessarily the same. Such a defect might be responsible for the slow-growth phenotypes of these *rpoB* mutants (9). At present, we do not know which step in initiation is defective at these promoters for the mutant RNAPs. The four *rpoB* mutations are located adjacent or close to each other within the rif-region in the β subunit of RNAP. Apparently, the sites in RNAP, which are affected by these *rpoB* mutations, are important for the interaction between RNAP and the stringent promoters. Other important functions, such as promoter clearance, elongation, and termination, also are attributed to the rif-region of RNAP (23).

Coupling of Transcription and the Stability of Initiation Complexes at Stringent Promoters. Our study indicates that the rate-limiting step in transcription of stringent promoters is the stability of RNAP·promoter complexes. We speculate that this feature is likely to be related to the regulation of this class of promoters in the cell, because we found a complete correlation between the *in vitro* and *in vivo* properties of these RNAP mutants. These RNAP mutants further destabilize the initiation complexes of stringent promoters *in vitro* and behave like "stringent" RNAPs *in vivo* in the absence of the stringent response. The rate of total RNA synthesis was about 2-fold lower in the *rpoB114* mutant than that in the wild-type strain during exponential growth (7), indicating that the RNAP mutant has reduced rRNA and tRNA syntheses *in vivo* (also see Table 1). Such a correlation indicates strongly that destabilizing the initiation complexes at stringent promoters is responsible for the stringent response.

Based on our results, we propose a model for the regulation of stringent promoters (Fig. 7). According to this model, the stability of initiation complexes at this class of promoters can change from a relatively stable state with supercoiled to a very unstable state with completely relaxed DNA, with intermediate metastable states in between these two extremes. This is because the initial steps before the first phosphodiester bond formation are reversible with the intermediates being in rapid equilibrium with each other at this class of promoters. In contrast, the initiation complexes at nonstringent promoters are very stable in general because of the irreversible nature of the isomerization step (24). Because transcription and supercoiling of the DNA template are interrelated (25, 26) and transcription elongation causes a local increase in negative superhelicity upstream of the transcribing RNAP, a high rate of initiation increases the superhelicity of a promoter. Therefore, we propose that transcription activity of the stringent promoters would feed back to the stability of initiation complexes at these promoters through modulation in superhelicity. This unique coupled feature is manifested at stringent promoters because the stability of the initiation complexes of this class of promoters is a rate-limiting step and is very sensitive to the superhelicity of DNA template. Thus, during rapid growth, transcription of stringent

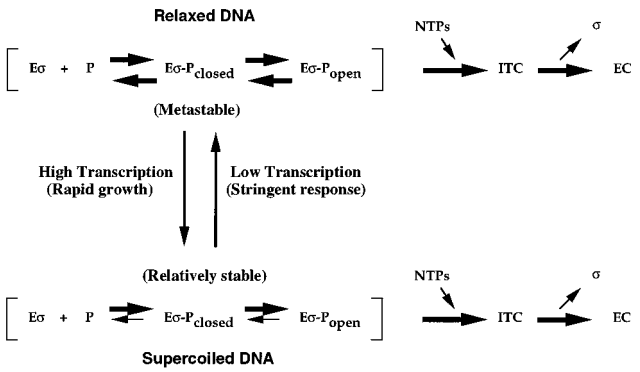

FIG. 7. A model of "transcription and the stability of initiation complexes are coupled at stringent promoters." ITC, initially transcribing complex. EC, elongation complex. For details, see text.

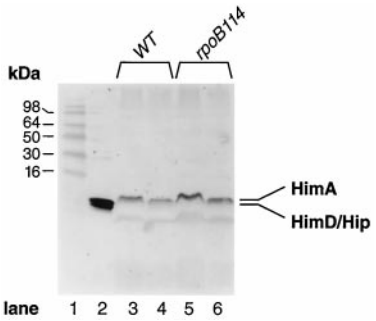

FIG. 8. The levels of IHF in wild-type (WT) and the *rpoB114* mutant in the absence of the stringent response. IHF in exponentially grown cells were analyzed as described in *Materials and Methods*. The two subunits of IHF were indicated. Lane 1, size marker; lane 2, purified IHF; lane 3 and 4, wild type, but the amount of cells in lane 4 was 2-fold less than that in lane 3; lane 5 and 6, *rpoB114*, but the amount of cells in lane 6 was 2-fold less than that in lane 5. The cells in lane 3 and 5 were equal OD_{600} units. Two different amounts of cells were used to ensure reproducibility.

promoters is highly active (27, 28), which is facilitated by making the initiation complexes at these promoters more stable. However, during amino acid starvation leading to the stringent response, transcriptional activity at these promoters is reduced. This causes a local reduction in superhelicity and destabilizes initiation complexes. Thus, a spectrum of the stability of initiation complexes will correspond to a range of transcription activity at these promoters at different growth rates.

The intrinsic instability of the initiation complexes at stringent promoters is correlated with the presence of a common sequence motif called "discriminator," which is GC rich between the −10 and +1 position, at these promoters (29–31). The "discriminator" sequence might provide a high-energy barrier that decreases the stability of the initiation complexes by reducing the isomerization rate. In the proposed model, DNA supercoiling overcomes the energy barrier, thus stabilizing the initiation complexes and increasing open complex formation (32).

The ppGpp molecule is closely associated with the stringent response (33, 34) and is very likely the metabolic signal for triggering the response (1). ppGpp could decrease either directly the stability of initiation complexes of stringent promoters (32, 35, 36), or indirectly by decreasing the transcription activity in general (37, 38). This in turn could decrease the stability of the initiation complexes at this class of promoters, as proposed in the model. The multiple effects of ppGpp are consistent with our model, although further study of the precise role of ppGpp in this process is clearly necessary.

This model also could explain how dual aspects of the stringent response are mechanistically related. We propose that the rate-limiting step for transcription initiation at the promoters that are positively controlled by stringent response is RNAP binding, making these promoters very sensitive to the concentration of free RNAP molecules. In contrast, binding of RNAP to stringent promoters (negatively controlled by the stringent response) is efficient (ref. 17; M. Fisher and D.J.J., unpublished results), making them relatively insensitive to the changes in RNAP concentration. During rapid growth, most RNAP molecules inside the cell are actively engaged in transcription of stringent genes (27, 28). Therefore, the level of free RNAP molecules would be limiting for the genes positively controlled by the stringent response. During the stringent response, or the "mimic stringent response" in the RNAP mutants described in this study, transcription of stringent genes is greatly reduced because most RNAP molecules are dissociated from these promoters (Fig. 7). Consequently, more RNAP molecules are available to initiate transcription at the genes that are positively controlled by the stringent response, leading to increased expression of these genes. In agreement with this model, we found that in the absence of the stringent response, the expression of the *himA* gene encoding the α subunit of IHF, which is positively controlled by the stringent response (39), is about 2-fold higher in *rpoB114* than in the wild-type strains (Fig. 8).

In essence, the stringent response is an important way to redistribute RNAP molecules to different sets of genes according to the growth conditions in the cell. Unlike in eukaryotes, a single RNAP is the sole enzyme responsible for all transcription activities in *E. coli*. Our study may elucidate a mechanism by which RNAP functions could be differentiated to achieve global regulation in response to environmental cue. Thus, our working model not only provides a simple mechanism for the regulation of the stringent response, but also can be tested experimentally in the future.

We thank Drs. Wilma Ross and Rick Gourse for plasmids and strains, Chuck Turnbough for plasmid, and Howard Nash for the antibody against IHF. We also thank Drs. Sankar Adhya, Mike Cashel, Tim Durfee, Sue Garges, Susan Gottesman, Carol Gross, and Bob Weisberg for their critical comments on the manuscript.

1. Cashel, M., Gentry, D. R., Hernandez, J. V. & Vinella, D. (1996) in *Escherichia coli and Salmonella, Cellular and Molecular Biology*, ed. Neidhardt, F. C. (Am. Soc. Microbiol., Washington, DC), Vol. 1, pp. 1458–1496.
2. Fiil, N. & Friesen, J. D. (1968) *J. Bacteriol.* **95,** 729–731.
3. Turnbough, C. L., Jr. (1983) *J. Bacteriol.* **153,** 998–1007.
4. Gourse, R. L., de Boer, H. A. & Nomura, M. (1986) *Cell* **44,** 197–205.
5. Ross, W., Thompson, J. F., Newlands, J. T. & Gourse, R. L. (1990) *EMBO J.* **9,** 3733–3742.
6. Liu, C., Heath, L. S. & Turnbough, C. L., Jr. (1994) *Genes Dev.* **8,** 2904–2912.
7. Zhou, Y. N. & Jin, D. J. (1997) *J. Bacteriol.* **179,** 4292–4298.
8. Jin, D. J. & Gross, C. A. (1988) *J. Mol. Biol.* **202,** 245–253.
9. Jin, D. J. & Gross, C. A. (1989) *J. Bacteriol.* **171,** 5229–5231.
10. Burton, Z. F., Gross, C. A., Watanabe, K. K. & Burgess, R. R. (1983) *Cell* **32,** 335–349.
11. Lupski, J. R., Smiley, B. L. & Godson, G. N. (1983) *Mol. Gen. Genet.* **189,** 48–57.
12. Grossman, A. D., Taylor, W. E., Burton, Z. F., Burgess, R. R. & Gross, C. A. (1985) *J. Mol. Biol.* **186,** 357–365.
13. Xiao, H., Kalman, M., Ikehara, K., Zemel, S., Glaser, G. & Cashel, M. (1991) *J. Biol. Chem.* **266,** 5980–5990.
14. Miller, J. H. (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Lab. Press, Plainview, NY).
15. Jin, D. J. (1996) *J. Biol. Chem.* **271,** 11659–11667.
16. Hager, D. A., Jin, D. J. & Burgess, R. R. (1990) *Biochemistry* **29,** 7890–7894.
17. Gourse, R. L. (1988) *Nucleic Acids Res.* **16,** 9789–9809.
18. Leirmo, S. & Gourse, R. L. (1991) *J. Mol. Biol.* **220,** 555–568.
19. Pfeffer, S. R., Stahl, S. J. & Chamberlin, M. J. (1977) *J. Biol. Chem.* **252,** 5403–5407.
20. Donahue, J. P. & Turnbough C. L., Jr. (1990) *J. Biol. Chem.* **265,** 19091–19099.
21. Jin, D. J. & Turnbough, C. L., Jr. (1994) *J. Mol. Biol.* **236,** 72–80.
22. Heisler, L. M., Feng, G., Jin, D. J., Gross, C. A. & Landick, R. (1996) *J. Biol. Chem.* **271,** 14572–14583.
23. Jin, D. J. & Zhou, Y. N. (1996) *Methods Enzymol.* **273,** 300–319.
24. McClure, W. R. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 5634–5638.
25. Liu, L. F. & Wang, J. C. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 7024–7027.
26. Wang, J. C. & Lynch, A. S. (1993) *Curr. Opin. Genet. Dev.* **3,** 764–768.
27. Ingraham, J. L., Maalfe, O. & Neidhardt, F. C. (1983) *Growth of the Bacterial Cell* (Sinauer, Sunderland, MA), pp. 349–385.
28. Nomura, M., Gourse, R. L. & Baughman, G. (1984) *Annu. Rev. Biochem.* **53,** 75–117.
29. Travers, A. A. (1980) *J. Bacteriol.* **41,** 973–976.
30. Travers, A. A. (1980) *J. Mol. Biol.* **141,** 91–97.
31. Zacharias, M., Goringer, H. U. & Wagner, R. (1989) *EMBO J.* **8,** 3357–3363.
32. Ohlsen, K. L. & Gralla, J. D. (1992) *Mol. Microbiol.* **6,** 2243–2251.
33. Cashel, M. (1969) *J. Biol. Chem.* **244,** 3133–3141.
34. Cashel, M. & Gallant, J. (1969) *Nature (London)* **221,** 838–841.
35. Hamming, J., Ab, G. & Gruber, M. (1980) *Nucleic Acids Res.* **8,** 3947–3963.
36. Kingston, R. E., Gutell, R. R., Taylor, A. R. & Chamberlin, M. (1981) *J. Mol. Biol.* **146,** 433–449.
37. Vogel, U. & Jensen, K. F. (1994) *J. Biol. Chem.* **269,** 16236–16241.
38. Kingston, R. E. & Chamberlin, M. J. (1981) *Cell* **27,** 523–531.
39. Aviv, M., Giladi, H., Schreiber, G., Oppenheim, A. B. & Glaser, G. (1994) *Mol. Microbiol.* **14,** 1021–1031.