Effect of Varying the Supercoiling of DNA on Transcription and Its Regulation

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ABSTRACT: The effect of superhelicity of DNA templates on transcription is well documented in several cases. However, the amount of supercoiling that is needed to bring about any changes and the steps at which such effects are exerted were not systematically studied. We investigated the effect of DNA supercoiling on transcription from a set of promoters present on a plasmid by using a series of topoisomers with different superhelical densities ranging from totally relaxed to more than physiological. In vitro transcription assays with these topoisomers in the absence and presence of gene regulatory proteins showed that the effect of negative supercoiling on intrinsic transcription varies from promoter to promoter. Some of those promoters, in which DNA superhelicity stimulated transcription, displayed specific optima of superhelical density while others did not. The results also showed that the amounts of abortive RNA synthesis from two of the promoters decreased and full-length RNA increased with increasing supercoiling, indicating for the first time an inverse relationship between full-length and abortive RNA synthesis and supporting a role of DNA superhelicity in promoter clearance. DNA supercoiling might also influence the point of RNA chain termination. Furthermore, the effect of varying the amount of supercoiling on the action of gene regulatory proteins suggested the mode of action, which is consistent with previous results. Our results underscore the importance of DNA supercoiling in fine-tuning promoter activities, which should be relevant in cell physiology given that local changes in chromosomal supercoiling must occur in different environments.

Transcription of many genes, like other DNA transactions, is affected by DNA supercoiling (*1*−*3*). Negative supercoiling of the DNA template is usually more efficient for transcription (*4*−*9*). At the macroscopic level, gene transcription has several steps: RNA polymerase occupancy at the promoter, isomerization, promoter clearance, elongation, and termination as well as regulation of these processes by DNA binding regulatory proteins. Intrinsic and guided interactions between segments of DNA, domains of RNA polymerase, and regulatory proteins must take place during various steps of transcription. The following observations previously suggested that negative DNA supercoiling affects one or more of these steps in the promoters studied. (i) RNA polymerase and regulatory proteins may prefer binding to supercoiled DNA (*10*−*12*). (ii) DNA is underwound in open and elongating complexes (*13*−*16*), and underwinding is favored by negative supercoiling (*17*, *18*). Supercoiling indeed enhances isomerization in the bacteriophage λP_L and *lacP*^{s} promoters (*19*, *20*). Interestingly, supercoiling inhibits isomerization in the $lacP_{uv5}$ promoter (*11*). (iii) The step of promoter clearance may also be subject to the influence of DNA supercoiling (*21*, *22*). (iv) A pause in transcription elongation was shown to be enhanced by DNA supercoiling (*23*). (v) Supercoiloing affects transcription termination (*24*). These results and the twin-domain supercoiling of a transcribing DNA segment, accumulation of positive superhelicity ahead of RNA polymerase and negative superhelicity behind it (*3*, *25*, *26*), strongly implicate DNA in playing an active rather than a passive role in gene transcription. As expected, the level of transcription of many genes is dependent upon the level of DNA gyrase and DNA topoisomerse I in vivo (*21*, *22*, *27*−*31*). In fact, the activity of DNA gyrase helps some promoters while inhibiting others (*22*, *32*). Promoters were found that are insensitive to DNA supercoiling (*22*, *31*). However, the amounts of supercoiling were not varied when studying the effect of supercoiling on the steps of transcription in different promoters in vitro. Besides, the effects on different promoters could not be compared quantitatively because of different experimental setups. We initiated a detailed investigation of the effect of varying the amount of negative supercoiling of DNA on intrinsic and regulated in vitro transcription in a set of *Escherichia coli* promoters present in the same plasmid and compared their response quantitatively. We generated a series of topoisomers of the plasmid that harbors these promoters and studied the effect of DNA topology on in vitro transcription from these promoters in the absence and presence of their cognate regulatory proteins. In this first communication, we report both the qualitative and quantitative effects of different amounts of negative supercoiling on the overall RNA synthesis, abortive transcription (when relevant), and the effects of any regulatory protein on these promoters. The results clearly showed that the effect of DNA

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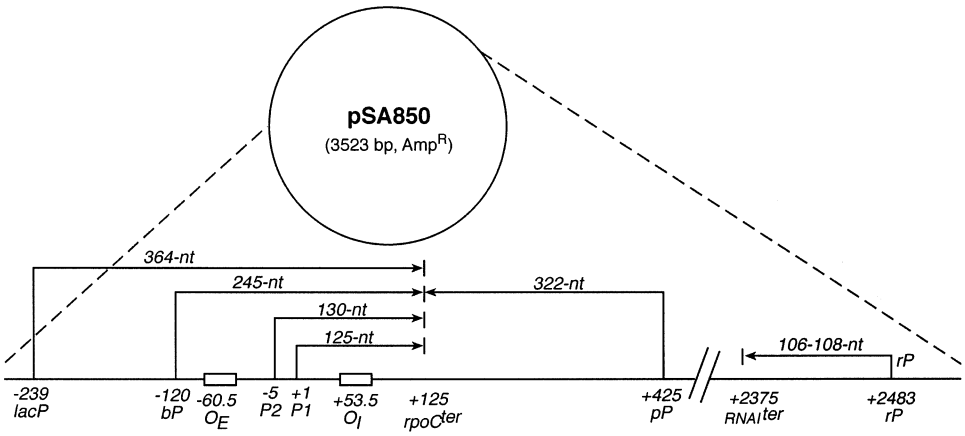

FIGURE 1: Orientation and location of the various promoters and terminators, discussed in the text, in the plasmid pSA850. The locations refer to numbers denoting the start sites of transcription of the corresponding promoters with respect to the start site of *galP1* (designated +1). The promoter symbols are in the text, except that *P1* and *P2* refer to *galP1* and *galP2*, respectively, and O_E and O_I to the two GalR binding sites. The map is not drawn to scale.

supercoiling on transcription and regulator action depended on the nature of the promoter and the activity of the regulator. The optimal superhelical density required for intrinsic transcription and regulator action varied from promoter to promoter. We discuss the target step(s) of DNA supercoiling in affecting transcription at these promoters.

EXPERIMENTAL PROCEDURES

Plasmids and Proteins. The plasmid pSA850 contains several promoters followed by a transcription terminator to produce transcripts of discrete sizes (Figure 1). They are the promoter of the *lac* operon (*lacP*), the two overlapping promoters of the *gal* operon (*galP1* and *galP2*), a promoter (*pP*) located at the attachment region (*P′OP*)[1] of bacteriophage *λ*, and a promoter (*bP*) located in the bacterial attachment region (*B′OB*), which recombines with the *λ P′OP* site for integration of the phage genome into bacterial genome (*33*). In addition, pSA850 also contains the plasmid *rep* promoter (*rP*) with its own terminator (see below). Plasmid DNA were purified by CsCl density gradient ultracentrifugation and used as templates for in vitro transcription reactions.

GalR was purified from an *Escherichia coli* strain harboring a plasmid carrying the *galR* gene (pAM2) as described (*34*). CRP, purified to homogeneity by a final FPLC column, was a gift from Susan Garges and Thomas Soares (Laboratory of Molecular Biology, NCI, NIH). HU was purified as described in ref *35*. The RNA polymerase holoenzyme of *E. coli* was purchased from Pharmacia.

Preparation of Topoisomers of pSA850. Six micrograms of pSA850 was incubated at 37 °C in a 100 μL volume containing 24 units of calf thymus topoisomerase I (BRL) in 1× topoisomerase buffer and different amounts (0−6.4 μg/mL) of ethidium bromide in 100 μL of the topoisomerase buffer (BRL) at 37 °C for 2 h. After the reactions, proteins and ethidium bromide were removed by a phenol−chloroform (1:1) extraction and two successive isoamyl alcohol extractions. DNA was precipitated with ethanol. The average linking number difference (ΔLk) of the generated topoisomers was measured by using either 0.5 or 2.5 μg/mL of chloroquine in TAE-buffered (1% or 2%) agarose gel (*36*, *37*). The superhelical densities (σ) of the topoisomers were determined as described by Singleton and Wells (*38*). Using the number 10.5 bp/helical turn for B-DNA (*39*), the linking number (Lk) of totally relaxed pSA850 (3523 bp) was calculated to be 332.

In Vitro Transcription. In vitro transcription reactions were performed at 37 °C in a 50 μL volume. The initial mixture (45 μL) contained 2 nM plasmid DNA template, 20 nM RNAP, 20 mM Tris−acetate, pH 7.5, 10 mM magnesium acetate, 200 mM potassium glutamate, and 40 units of rRNasin (Promega). When required, GalR was present at 80 nM, HU at 80 nM, CRP at 50 nM, and cAMP at 100 μM, respectively. The initial mixtures were incubated for 5 min at 37 °C before 5 μL of NTP (2 mM of ATP, GTP, and CTP, 0.2 mM UTP, and 5 μCi of [α-^{32}P]UTP) was added to start the reactions. They were further incubated at 37 °C for 10 min. The reactions were terminated by the addition of 50 μL of the STOP solution (BRL). The samples were heated to 90 °C for 2 min, and 3 μL of each sample was loaded onto 8% and 25% polyacrylamide−urea sequencing gels to monitor the full-length transcripts and aborted molecules made from the promoters, respectively. Quantification of the transcripts was performed on PhosphoImager.

RESULTS

DNA Topoisomers of Varying Negative Superhelicity. A series of topoisomers with an average superhelical density (σ) from 0 to −0.093 (linking number difference, ΔLk, from 0 to −31) of the plasmid pSA850 containing several promoters were generated as described in Experimental Procedures. The distribution of the topoisomers made in the presence of varying ethidium bromide concentrations is shown in Figure 2. The superhelical density σ of the pSA850 population isolated from *E. coli* cells was −0.062 (ΔLk = −21). Thus, the state of the topoisomers ranged from totally relaxed to more than physiologically supercoiled. In vitro transcriptions were performed using the topoisomers as DNA templates to analyze supercoiling dependency of the different promoters in pSA850 in the absence and presence of regulatory proteins. The results of RNA synthesis are shown

[1] Abbreviations: *B′OB*, bacterial attachment region for prophage *λ*; CRP, cAMP receptor protein; αCTD, α carboxy-terminal domain; αNTD, α amino-terminal domain; Lk, linking number; *P′OP*, *λ* attachment site to bacteria.

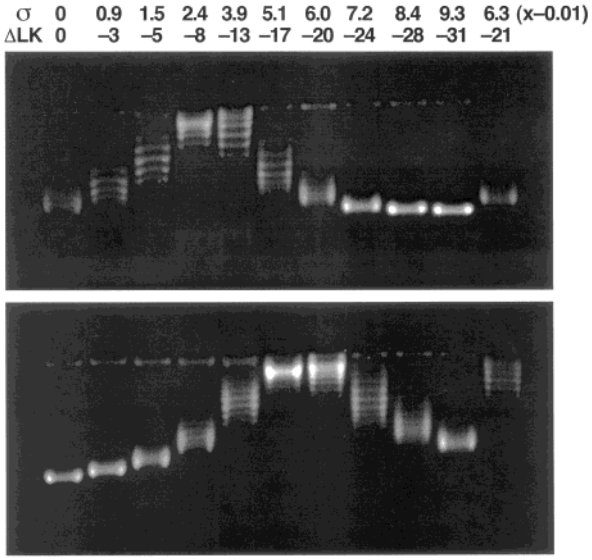

FIGURE 2: Topoisomers of the plasmid pSA850 generated as described in the text and used in the transcription assays. The topoisomers were separated by agarose gel electrophoresis in the presence of 0.5 mg/mL (upper gel) and 2.5 mg/mL (lower gel) of chloroquine. The average superhelical density (σ), and the linking difference (ΔLk) of each topoisomer are shown.

in Figures 3, 4, and 6. The quantification of some of the results is shown in Figure 5 and in Table 1.

Effect of Supercoiling on Intrinsic Transcription. As shown in Figure 3, the plasmid generated a large number of transcripts from the six identified promoters, *lacP*, *galP1*, *galP2*, *rP*, *bP*, and *pP* (see Experimental Procedures). Since all of the promoters, except *rP*, shared the same terminator (*rpoC* terminator, which is bidirectional) in the plasmid template (*33*), the observed differential effects of DNA supercoiling on the five promoters were at the level of one or more steps of initiation and not at the level of termination. The case of *rP* is discussed separately. It was clear that the superhelical density requirement for optimal transcription initiation varies from promoter to promoter under defined conditions. For example, RNA synthesis from the *pP* promoter, whose intrinsic strength was the highest among the set, was independent of the superhelical state of the template; there was no difference in the amount of RNA made whether the DNA was relaxed or supercoiled by any amounts. On the other hand, as the supercoiling increased, there was an increase in the synthesis of the amount of RNA made from the remaining four promoters. The transcription efficiency increased with the introduction of increasing amounts of negative density in the templates. Clearly, higher superhelical density decreased transcription from some of them, thus showing an optimum, which also varied from promoter to promoter. The intrinsic transcription from the paradigm *lacP* promoter increased continuously from a very low level at $\sigma = 0$ to 2.9-fold at $\sigma = -0.093$ without an optimum (Figures 4a and 5a, Table 1). The two *gal* promoters, *galP1* and *galP2*, although separated by 5 bp (*40*), responded quite differently from each other by DNA superhelicity changes. The amount of full-length RNA made from *galP1* increased gradually, but marginally, by changing the σ from 0 to -0.093, with no optimum as in *lacP* (Figures 4c and 5b, Table 1). On the other hand, transcription from *galP2* peaked around the superhelical density of $\sigma = -0.062$

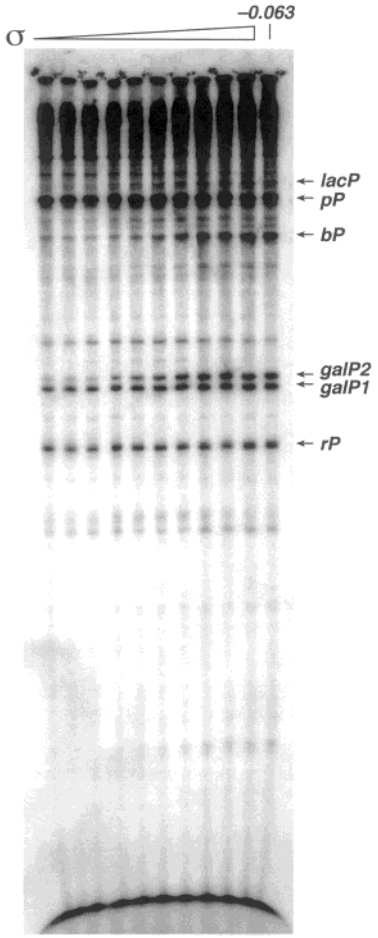

FIGURE 3: In vitro RNA synthesis as separated by gel electrophoresis from the different topoisomers of pSA850 DNA. An 8% gel, which can resolve full-length transcripts, made from the promoters in pSA850 was used. The increase of superhelical density (σ) is the same as shown in Figure 2. The origin of the identified transcripts are shown by arrows.

(Figures 4c and 5c, Table 1). The *galP2* produced about 6-fold more transcripts at the optimal density than at $\sigma = 0$ (fully relaxed), while *galP1* showed only a 2.25-fold increase from $\sigma = 0$ to the highest density, demonstrating that transcription from *galP2* was more dependent on negative supercoiling than that from *galP1*. The *rP* promoter mostly showed a single start site in our transcription system, as determined by primer extension assays, which resolved into two RNA products differing by one nucleotide residue (UMP) at the 3′ end because of variability in the exact point of termination in the Rho-independent terminator, which is cognate to *rP* (*41*; M. Liu, unpublished results). The effect of changing DNA superhelicity on RNA made from *rP* is discussed later. As shown in Figure 3, the dependencies of transcription on changing negative supercoiling of DNA template were also true for several other unidentified promoters present on the plasmid and were not studied further.

Effect of Changing DNA Superhelicity on the Activities of Gene Regulatory Proteins. Four of the promoters studied, *lacP*, *galP1*, *galP2*, and *pP*, are regulated; no regulators are known for the other two, *rP* and *bP*. We studied the effects of DNA supercoiling on the CRP effect in *lacP* and the CRP, GalR, and HU effects in the two *gal* promoters. In vitro transcription assays were performed with the topoisomers

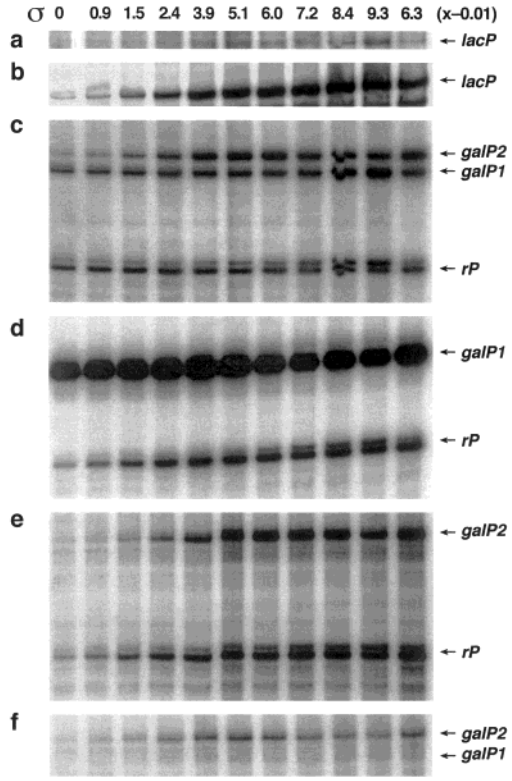

FIGURE 4: Electrophoretic separation of full-length transcripts made from *lacP* in the presence (a) or in the absence of CRP (b) and the *gal* promoters without any factors (c) or in the presence of CRP (d), GalR (e), or GalR and HU (f) at different superhelical densities of DNA as indicated. Transcripts are identified by arrows on the right (see Figure 1).

in the presence of CRP as shown in Figures 4 and 5. The regulation of *pP* by its regulator (IHF) was not studied.

(i) Activation of *lacP*. CRP, which regulates the *lacP* promoter by binding to a DNA site centered at the −61.5 position (*42*), stimulated transcription at all superhelical densities from zero to the highest. The stimulation was 5-fold at zero superhelicity and 55-fold at $\sigma = -0.093$ (Figure 5, Table 1).

(ii) Regulation of *galP1* and *galP2*. The *gal* promoters are controlled by three transacting proteins, GalR, CRP, and the nucleoid protein HU (*43*−*48*). GalR binds to two operator DNA sequences, O_E (centered at −60.5) and O_I (centered at +53.5). GalR requires HU to simultaneously repress both *galP1* and *galP2* promoters. Besides the concurrent repression of the two promoters, GalR binding to O_E alone brings about repression of *galP1* and enhancement of *galP2*. The control of *galP1* and *galP2* by CRP is the opposite of that by GalR alone. CRP binds to a DNA site at position −41.5 and represses *galP2* and enhances *galP1*. (a) Repression: CRP-mediated repression of *galP2* and GalR-mediated repression of *galP1* occurred regardless of the superhelical nature of the DNA template (Figure 4d,e); the amount of repression of *galP1* and *galP2* by the corresponding regulators occurred uniformly from zero to the highest supercoiling density. (b) Activation: Unlike the repression, the activation of *galP1* by CRP and of *galP2* by GalR changed with different superhelical densities (Figure 4d,e). The maximum CRP activation (12-fold) on *galP1* (Figure 5b, Table 1) was achieved at the σ of −0.051 with decreased activation at higher densities. This contrasted to the finding that there was

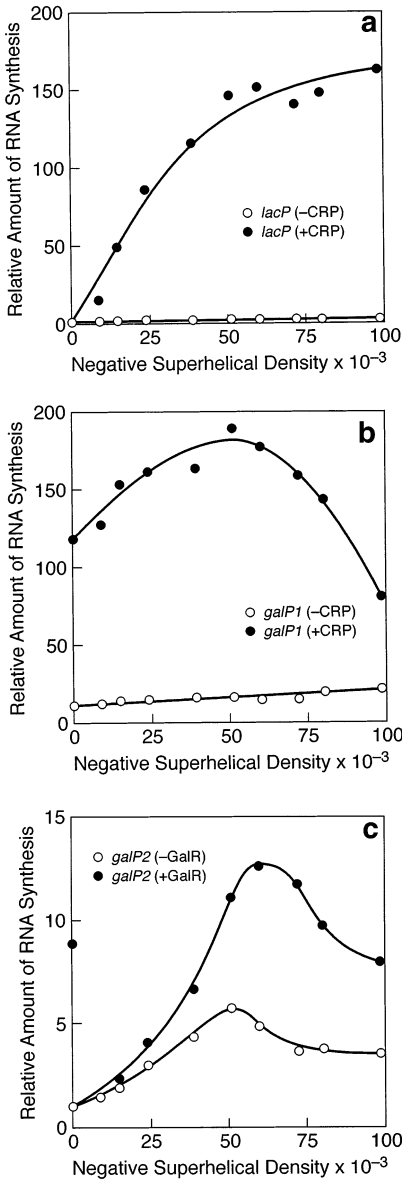

FIGURE 5: Quantification profiles of the full-length transcripts made from the *lac* promoter (a), *galP1* promoter (b), and *galP2* promoter (c). The symbol designations are shown.

no optimum in *galP1* in the absence of CRP. The activation of *galP2* by GalR at O_E seemed to be affected more by negative supercoiling than that of *galP1* by CRP. Activation of *galP2* by GalR (Figures 4e and 5c, Table 1) did not occur when the template was fully relaxed. The activation increased exponentially with increasing superhelical density of the template and maximized at the physiological σ of −0.062, a slight increase from the optimal density corresponding to the intrinsic transcription (−0.051). Unlike GalR in *galP2*, the activation of *galP1* by CRP occurred even when the plasmid was fully relaxed (Figure 5b). (c) DNA looping mediated repression: In the presence of both GalR and HU, the *gal* promoters were repressed, as expected, concurrently (Figure 4f). The *galP2* repression by GalR and HU, which occurs upon DNA loop formation by interaction between two GalR dimers bound to O_E and O_I, required supercoiled DNA template (*48*, *49*). Therefore, it was critical to determine the extent of this dependency by using the topoisomers of varying superhelicity by comparing the *galP2*

Table 1: Transcription Stimulation by DNA Supercoiling[a]

promoter	superhelicity	relative transcription −CRP	+CRP	+GalR
lacP	0	1.0	5.0	
lacP	0.093	2.96	164.5	
galP1	0	1.0	11.8	NQ[b]
galP1	0.051	2.18	19.0	NQ
galP2	0	1.0	NQ	1.04
galP2	−0.060	5.74	NQ	12.67
bP	0	1.0		
bP	−0.093	10.0		
pP	0	1.0		
pP	−0.093	1.0		

[a] Relative amounts of RNA made from the *lac* and *gal* promoters in the presence of regulatory proteins with fully relaxed DNA templates or with DNA templates at the corresponding superhelicity for maximal transcription. The values are expressed relative to the corresponding amount of intrinsic transcription of a promoter with relaxed template. [b] NQ, not quantified.

transcription between that in the presence of GalR only (caused *galP2* activation) and GalR and HU (repression). The DNA looping mediated repression started to become obvious at a σ of −0.015 and showed an optimum at the physiological density of −0.063.

Abortive Transcription vs Changing DNA Superhelical Density. Having seen that the amount of full-length transcripts of the *gal* promoters increased with increasing superhelical density of the template, we were interested in the changes that might occur in the amount of the abortive transcripts from these promoters with increasing supercoiling. Thus, the in vitro transcription reactions of Figure 4 were analyzed on 25% acrylamide gels where abortive transcripts become visible (Figure 6). Interestingly, almost all of the major abortive transcripts observed decreased with increasing superhelical density. We identified some of the aborted RNA molecules in our system to be of *gal* origin by comparing the identities, the sizes, and the appearance and disappearance of the abortive transcripts in the absence and presence of CRP (*47*; unpublished observations). Overall, the abortive transcripts from *galP1* gradually decreased by 5-fold and those from *galP2* decreased 4-fold when the σ changed progressively from 0 to −0.093.

DNA Superhelicity and Point of Transcription Termination. As mentioned, the *rP* promoter made two RNA molecules with the same 5′ end but differing by one UMP residue at the 3′ end. The ratio of the amounts of the two RNA species depended upon the UTP concentrations in the transcription reactions. At higher UTP concentrations, RNA with an extra UMP at the 3′ end was the predominant one; the reverse was true at lower UTP concentrations (data not shown). This is consistent with the observation of McDowell et al. (*41*) at several other Rho-independent terminators. At 0.2 mM UTP, the synthesis of both RNA species from *rP* was observed (Figure 4). The longer one was made in the highest amount at $\sigma = -0.093$, whereas the synthesis of the shorter RNA peaked at $\sigma = -0.063$, alluding to the fact that the amount of DNA supercoiling may influence transcription termination points.

DISCUSSION

Intrinsic activities of many promoters are known to be enhanced, inhibited, or unaffected by negative supercoiling of DNA in vivo (*1*−*3*). The in vitro results of transcription from a set of promoters under the same condition fit with this general concept. The results demonstrated the influence of changing negative supercoiling of DNA mainly on several aspects of transcription initiation. Negative superhelicities showed a differential effect on transcription from several promoters but did not have any effect on another present on the same DNA template molecule. The effects of changing DNA supercoiling on the intrinsic and regulated transcription initiation, as well as on the abortive transcription, and the observed difference in the optima of supercoiling densities in these processes depended on the nature of the promoter. These results, summarized below, suggest that the DNA sequence difference in the promoters is the primary determinant of the observed differential effects of DNA superhelicity on transcription from these promoters.

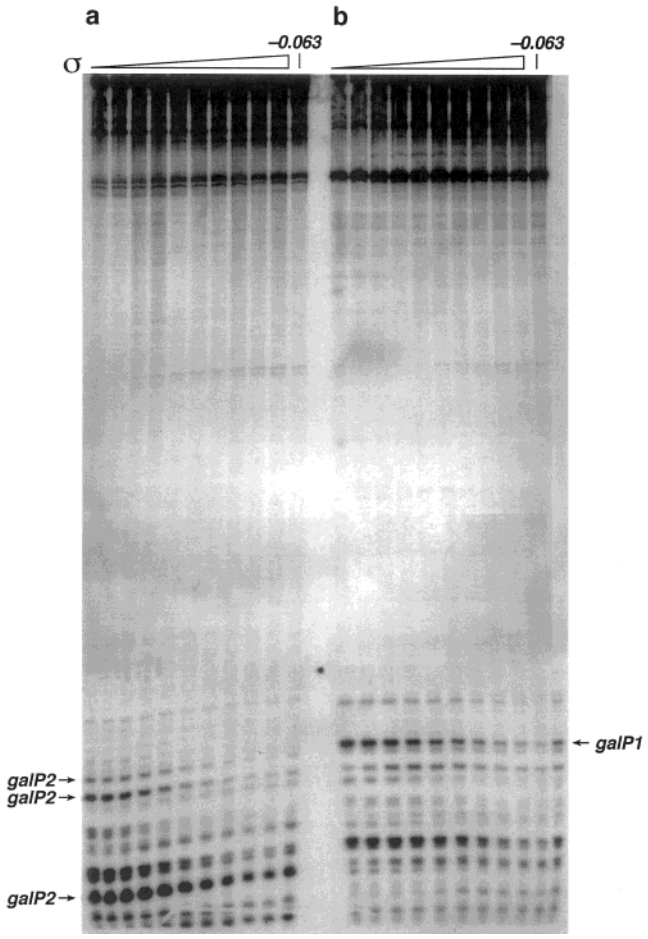

FIGURE 6: Electrophoretic separation of aborted RNA made from the *gal* promoters in the different topoisomers in a 25% sequencing gel. The increase of superhelical density (σ) is same as in Figure 2. The identified abortive RNA species are indicated by arrows in the margins. Transcription in the (a) absence and (b) presence of CRP.

pP. The transcription of the λ promoter, whose physiological significance is unknown and whose in vitro strength was very high, was unaffected by DNA supercoiling. These suggest that the DNA sequence in this promoter has been optimized so as not to depend on negative supercoiling.

bP. The intrinsic strength of the bacterial promoter located at the host attachment site for prophage λ insertion was very weak and activated considerably (10-fold) by DNA super-

coiling. The transcription initiation step at which DNA superhelicity affects *bP* remains to be investigated.

rP. The *rP* promoter is already known to be stimulated by DNA supercoiling at the level of isomerization (*50*). However, RNA made from this promoter terminated at two different points, and the effect of changing DNA supercoiling was different on the two RNA species. Thus, supercoiling may also affect the precise end point of the terminator of *rP*. It is not surprising since termination is a process in which there is a sequence-dependent interplay between RNA polymerase release, DNA unwinding, and DNA collapsing.

lacP. CRP stimulated the intrinsic transcription at the *lacP* 5-fold on relaxed DNA, whereas DNA supercoiling alone (at the maximal density) increased transcription 2.9-fold (Table 1). The CRP stimulation on the supercoiled DNA was synergistic (164-fold). The very low level of intrinsic transcription of the paradigm *lacP* promoter was attributed to defects both in RNA polymerase binding and in isomerization (*12*). CRP helps the enzyme occupancy by a contact between CRP and αCTD of RNA polymerase (*12*, *51*). While DNA supercoiling may be stimulating RNA polymerase binding, the highly synergistic stimulation of *lac* transcription on supercoiled DNA in the presence of CRP suggests that supercoiling was highly synergistic; supercoiling is likely affecting one or more steps beyond binding, isomerization and/or promoter clearance. This proposal is currently being studied.

galP1 and *galP2*. The intrinsic transcription of both *galP1* and *galP2* is of moderate strength. Since supercoiling helped intrinsic transcription of *galP1* 2.2-fold at the highest superhelical density, the observed CRP stimulation of 12-fold with relaxed DNA and 19-fold with the optimally supercoiled DNA suggests a cooperative effect of the two influences (Table 1). Note that CRP stimulates the *galP1* transcription efficiently at $\sigma = 0$ with about 50% more enhancement at the highest density. The cooperativity may indicate that the two stimulatory effects are independent. *galP1* is weak both in RNA polymerase binding and in isomerization (*52*), and different domains of CRP improve the two initiation steps by contacting with the αCTD and αNTD of RNA polymerase, respectively (*53*). The exact step at which DNA supercoiling affects transcription at *galP1* needs to be determined.

The intrinsic transcription of the *galP2* promoter was previously shown to be enhanced by DNA supercoiling (*48*, *49*). The current observations confirmed that and further showed that supercoiling-mediated optimal activation occurred at the density of $\sigma = -0.051$, which is slightly lower than the physiological density. GalR stimulated *galP2* only when DNA is significantly supercoiled with the optimum switching to $\sigma = -0.062$ (Figure 5c, Table 1). Since GalR acts by stimulating the step of isomerization by making a contact with αCTD (*54*), supercoiling is likely helping DNA unwinding by facilitating a specific angular orientation between DNA-bound GalR and αCTD. We are further investigating the biochemical mechanisms by which GalR acts.

Since the repression of *galP1* and *galP2* by GalR and CRP, respectively, is independent of the superhelical state of DNA, the two repression activities must be occurring at steps not involving DNA unwinding. We discussed above that RNA polymerase binding at these two promoters does not need supercoiling whereas isomerization is enhanced by it. This suggests that the repression effect is at a level between binding and isomerization. Consistently, GalR-mediated repression of *galP1* transcription indeed occurs at such an intermediate step (*54*). The level at which CRP acts to repress *galP2* is unknown.

DNA Looping. In the presence of HU, GalR dimers bound on O_E and O_I bring the two operators together to form a DNA loop (*35*, *48*). HU binds to a DNA sequence centered at +6.5. Given that a contact between two DNA-bound GalR molecules for DNA looping at the *gal* promoters is weak (*55*), a stable contact between them needs both DNA bending and untwisting of the segment between the two operators (*56*, *57*). The bending requirement is met by binding of the DNA-bending protein HU (*55*, *58*). Structural simulations predicted that the untwisting part should be facilitated by DNA supercoiling (*56*). Our supercoiling titration results confirm that prediction and further show that the loop is most stable when $\sigma = -0.062$, the physiological density.

Promoter Clearance. One revealing aspect of this study is the effect of DNA supercoiling on abortive RNA synthesis. The decrease in the synthesis of the abortive transcripts with increasing negative supercoiling was previously unknown. The synthesis of the abortive transcripts is known to be made by the open complexes, which do not clear the promoter. Both *galP1* and *galP2* made significant amounts of aborted RNA molecules at $\sigma = 0$, which progressively decreased with increasing superhelicity, suggesting that the promoter clearance step is limiting at these two promoters, and superhelicity of DNA overcomes the deficiency. Note that the full-length RNA synthesis reached an optimum at $\sigma = -0.051$, whereas the aborted RNA synthesis in *galP1* continued unabated at higher supercoiling density; very high supercoiling density may be inhibitory to the clearance step in *galP1*. Two models were proposed to account for the generation of the abortive RNA when observed in a promoter in vitro (*59*−*61*). (i) All of the RNA polymerase−promoter binary complexes go through the stage of abortive transcription before committing to clearance in such a promoter. If this model is correct, our results suggest that a lack of proper sequence-dependent DNA unwinding blocks promoter clearance, and negative supercoiling helps RNA polymerase to overcome the block. (ii) Only a fraction of the population of RNA polymerase−promoter binary complexes (moribund complexes) synthesizes abortive RNA molecules exclusively, the rest making full-length RNA (*59*). In this model, DNA supercoiling shifts the equilibrium of the binary complex population toward those that make productive RNA. In either case, how DNA supercoiling helps promoter clearance at the structural level would be an interesting question. Indeed, we note that there are specific promoters in which DNA supercoiling reduces promoter clearance (*21*).

In conclusion, our analysis of the effect of changing amounts of DNA superhelicity on transcription using a set of promoters, in the absence or presence of regulatory proteins, confirms that DNA supercoiling brings about another parameter to the steps of transcriptional regulation. Because of the occurrence of local changes in chromosomal supercoiling in vivo in different conditions (*62*), DNA supercoiling could delicately control expression of relevant genes located in those regions for physiological needs.

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