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Fusions of the *Escherichia coli gyrA* and *gyrB* Control Regions to the Galactokinase Gene Are Inducible by Coumermycin Treatment

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We have previously shown that the genes encoding the two subunits of *Escherichia coli* DNA gyrase are regulated in a manner which is dependent on DNA conformation. When the DNA encoding the *gyrA* and *gyrB* genes is relaxed, both genes are expressed at a high level; in negatively supercoiled DNA they are expressed at a low level. In this paper we describe fusions of both the *gyrA* and *gyrB* 5′ sequences to the *E. coli* galactokinase gene. In such fusions we found that galactokinase can be induced by treating the cells with coumermycin A1, an inhibitor of DNA gyrase. Our results suggest that the regulation occurs at the transcriptional level and that only a small region of DNA is necessary for coumermycin-induced gene expression.

Covalently closed DNA isolated from eubacteria is known to be negatively supercoiled (4, 9). If the supercoiling activity of DNA gyrase is blocked, cell growth is arrested (7, 11). Furthermore, the degree of supercoiling must be maintained in a suitable range. In *Escherichia coli*, studies have implied that excessive DNA supercoiling also inhibits cell growth (5, 18). It has been well documented that supercoiling is important to the DNA functions of replication, transcription, and certain types of recombination (see references 6 and 9 for reviews).

Given this background, we anticipated that there is some sort of control over the expression of the genes encoding DNA gyrase, the enzyme responsible for DNA supercoiling. Our initial studies demonstrated that the *gyrA* and *gyrB* genes are directly regulated by the level of DNA supercoiling, in a manner appropriate to the function of DNA gyrase. When DNA is relaxed, synthesis of the subunits of DNA gyrase increases, thus enhancing the capacity of the cells to supercoil their DNA. Conversely, when DNA is supercoiled, expression of the genes encoding DNA gyrase decreases.

From the standpoint of cellular regulation, the control of gyrase expression is readily understandable. However, the mechanism by which DNA relaxation activates transcription is not clear. Studies have demonstrated a mechanism for activation of transcription by DNA supercoiling. An early step in the initiation of transcription is the unwinding of the DNA by RNA polymerase to form the "open complex" (for a review see reference 13). This step is facilitated by negative supercoiling, which lowers the free energy needed to unwind DNA (8). If a promoter has a rate of initiation which is limited by the rate of open complex formation, one can expect an increased rate of initiation on a supercoiled DNA template. Numerous examples of genes whose expression is decreased in vivo by decreased supercoiling have been noted, and in at least two cases the effects have been reproduced with purified RNA polymerase and plasmid DNA and have been shown to alter the rate of open complex formation in the expected manner (2, 25). However, this mechanism cannot explain why a promoter is stimulated by DNA relaxation. Thus, such promoters are interesting objects of investigation.

As a next step in our study of the control of gyrase expression and the nature of relaxation-activated gene expression, we constructed operon fusions between the gyrase genes and the structural gene for galactokinase. These fusions and their behavior are described in this paper.

MATERIALS AND METHODS

Plasmids and strains. Plasmid pkO100 was obtained from Keith McKenney and is identical to plasmid pKO1 described by McKenney et al. (14) except that the *Hin*dIII-*Sma*I region has been replaced with a multisite *Hin*dIII-*Sma*I-*Bam*HI-*Eco*RI insertion, as shown in Fig. 1. Plasmids pMK47 and pMK90, which were used as sources of the *gyrB* and *gyrA* sequences, have been described by Mizuuchi et al. (16). Plasmid pKG1800, which was used as a control in the kinase assays, has been described by McKenney et al. (14). The plasmid DNA used in the cloning experiments described in this paper was prepared by the cleared lysate procedure and was banded twice in a CsCl-ethidium solution as previously described (22). The ethidium was removed by extraction with butanol, followed by exhaustive dialysis. The strains used in this study are listed in Table 1. The λ::Tn*9* vector used in the transposition analysis was a gift from Lee Rosner and has been described previously (19).

Bacteriological media. MacConkey agar plates, L medium, and minimal M63 plates were prepared as described by Silhavy et al. (20). Drugs were added as required from concentrated stocks (20 mg of sodium ampicillin per ml in water, resulting in a final concentration of 50 μg/ml; 80 mg of chloramphenicol per ml in ethanol, resulting in a final concentration of 20 μg/ml) after autoclaving. For most procedures cells were grown in L broth; for the galactokinase assay cells were grown in M63 medium containing 0.3% fructose and 20 μg of ampicillin per ml.

Transformation. Competent cells for transformation were grown overnight from a single colony in rich medium and then diluted 1:10 into fresh LB. Following growth for 2 h, the cells were chilled on ice, pelleted, and washed twice in cold (0°C) 50 mM $CaCl_2$. The washed cells were suspended to their original growth volume in the $CaCl_2$ solution and left to stand on ice for 1 h. These $CaCl_2$-treated cells were pelleted in the cold and suspended to a final volume that was equal to

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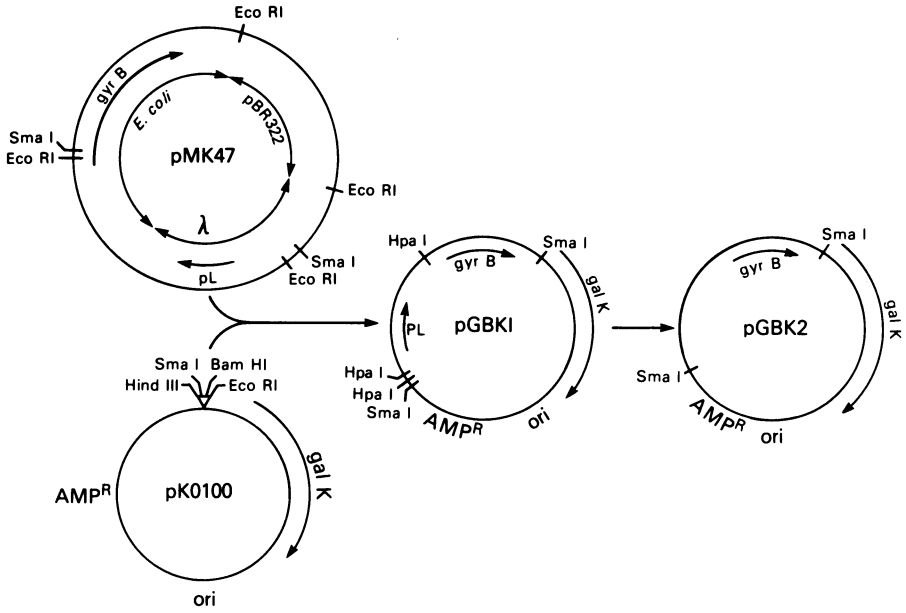

FIG. 1. In vitro fusion of *gyrB* to galactokinase. Details are given in the text. The origins of the sequence components of parental plasmid pMK47 are shown by the double-arrow segments. The rough positions and directions of transcription are indicated for *gyrB*, galactokinase (*galK*), and the λ p_L promoter by the single-arrow segments. Only the positions of the restriction sites relevant as landmarks to plasmid construction are given. When the restriction sites are indicated, all sites of that type on the plasmid are shown.

1/20th of their original growth volume in the cold $CaCl_2$ solution. DNA in TE (10 mM Tris, pH 8.0, 1 mM EDTA) was added to a portion of the cells so that the volume of the added DNA solution was less than or equal to 1/50th of the volume of the concentrated $CaCl_2$-treated cells. The cells and DNA were left on ice for 1 h and then rapidly heated to 37°C and left at 37°C for 5 min. The cells were immediately spread onto plates containing the appropriate drug.

TABLE 1. Bacterial strains used[a]

Strain	Plasmid	Description
RM264	pGBK2	Long *gyrB-galK* fusion plasmid without λp_L
RM267	pKO100	Vector alone
RM268	pGBK3	Short *gyrB-galK* fusion plasmid
RM269	pGBK1	Long *gyrB-galK* fusion plasmid with λp_L
RM270	pKG1800	Control *galOP-galK* fusion plasmid
RM280	pCA3	Other promoter *galK* (*gyrA* series)
RM281	pCA2	Inverse *gyrA-galK* fusion plasmid
RM299	pGAK1	*gyrA-galK* fusion plasmid
RM350	pRDC1	Random clone
RM351	pRDC2	Random clone
RM352	pRDC3	Random clone
RM353	pRDC4	Random clone
RM354	pRDC5	Random clone
RM355	pRDC6	Random clone
RM356	pRDC7	Random clone
RM357	pRDC8	Random clone
RM358	pRDC9	Random clone
RM359	pRDC10	Random clone
RM360	pRDC11	Random clone
RM361	pRDC12	Random clone
RM362	pRDC13	Random clone
RM363	pRDC14	Random clone
RM364	pRDC15	Random clone
RM365	pRDC16	Random clone
RM366	pRDC17	Random clone
RM367	pRDC18	Random clone
RM368	pRDC19	Random clone
RM369	pRDC20	Random clone

[a] The bacterial strains used in this study were derived from parental strain RM120 [*strR galK2* Δ(*srl recA*) *srl*::Tn*10*] by introducing plasmids which were isolated in this study.

Isolation of plasmid-borne Tn*9* insertions. Insertion element Tn*9* was transposed from the λ vector (cI857 *bio69 gam210 nin5* Tn*9*) into strain RM264 by using methods described by Rosner and Guyer (19). Pools of 2,000 chloramphenicol-resistant colonies were grown, and plasmid DNA was prepared. These plasmid preparations were then used to transform strain RM120 [*strR galk2* Δ(*srl recA*) *srl*::Tn*10*] to chloramphenicol resistance. The resulting chloramphenicol-resistant colonies were divided into Gal^+ and Gal^- collections, and the locations of the insertions were determined by restriction analysis.

Galactokinase assays. Cells for the galactokinase assay were grown to stationary phase from isolated single colonies in M63 medium containing 0.3% fructose and 20 μg of ampicillin per ml. These cultures were then diluted 1:1,000 in the same medium without ampicillin and grown to an optical density at 650 nm between 0.2 and 0.4 prior to the assay. The assays were performed essentially as described by McKenney et al. (14), with the following exceptions: [^{3}H]galactose (52 Ci/mmol; diluted to a final specific activity of 2 Ci/mol; New England Nuclear Corp.) was used rather than [^{14}C]galactose, and the assays were run for 20 min or less due to a loss of linearity at longer time intervals. All assays were run with both strain RM267 (plasmid pKO100, promoterless vector) and strain RM270 (plasmid pKG1800, wild-type *gal* control region, not coumermycin inducible) as controls. The value obtained for RM267 (<20 nmol of galactose phosphate per min per ml of a culture with an optical density at 650 nm of 1.0) was subtracted as the background level, and the corrected value was normalized to the activity of RM270 (350 to 550 nmol of galactose phosphate per min per ml of a culture with an optical density at 650 nm of 1.0), which was assigned a value of 1.00 in each

assay set. In our hands assay results presented in this way had levels of reproducibility of ±10% at high levels of activity; the level of reproducibility decreased to ±50% when the activity was only twice the background level. The levels of reproducibility in various experiments are given in the table footnotes. Poor reproducibility at low levels could lead to anomalous data (for example, the induction value of 0.27 for strain RM360 in Table 4). Assay results directly expressed as specific activities had greater variability. The results presented are averages for experiments done on at least 3 separate days. Although no effort was made to correct for plasmid copy number, in a few instances plasmid levels were compared by gel electrophoresis and staining with ethidium; the plasmid levels appeared to be the same. The plasmids used had the same sequence with the exception of a small insertion which directed the transcription of the galactokinase gene, and all inserted promoters had strengths which varied over a range of only fivefold; thus, we expected little variation in plasmid copy number.

For coumermycin induction a mid-log culture (see above) was split at zero time and either treated with coumermycin A1 (brought to a final concentration of 100 μg/ml from a 20-mg/ml stock in dimethyl sulfoxide) or mock treated with an equal volume of dimethyl sulfoxide alone. In control experiments dimethyl sulfoxide was shown to have no effect when it was added alone. Coumermycin A1 was purchased from Sigma Chemical Co.

DNA manipulations. Restriction enzymes, BAL31, mung bean nuclease, T4 polynucleotide kinase, and T4 DNA ligase were purchased from either New England BioLabs, Inc., or Bethesda Research Laboratories, Inc., and were used according to the recommendations of the suppliers. All BAL31 reactions were followed by mung bean nuclease treatment. For DNA sequence analysis plasmid pGBK3 was opened at the *Bam*HI site, treated with calf intestine phosphatase, labeled with T4 kinase, and digested with *Sau*3A1, and the appropriate labeled fragment was purified by polyacrylamide gel electrophoresis followed by electroelution. In one instance plasmid pGAK1 was cut at the *Eco*RI site and similarly labeled, a second cut was made at the *Hin*dIII site, and the appropriate piece was purified in a manner identical to that used for the *gyrB* fragment. Alternatively, plasmid pGAK1 was first cut and labeled at the *Hin*dIII site, and this was followed by a second cleavage at the *Eco*RI site. The sequence of plasmid pGAK1 was confirmed by determining the sequence of the insertion in plasmid pCA2 (inverse *gyrA*) by a completely analogous series of manipulations. All fragments were sequenced by the chemical method of Maxam and Gilbert (12).

RESULTS

Fusion of the *gyrB* 5′ sequences to *galK*. Plasmid pMK47 has been previously described as an expression vector for subunit B of DNA gyrase (16). In a previous paper we demonstrated that the *gyrB* protein was encoded as illustrated in Fig. 1 (10). Thus, the smaller DNA fragment extending from the *Sma*I site in the *E. coli* sequences of pMK47 to the *Sma*I site in the λ sequences contains the amino terminus of the *gyrB* protein, as well as *E. coli* sequences extending approximately 2 kilobases (kb) upstream and some λ sequences. This *Sma*I piece was prepared and inserted into the *Sma*I site of vector pKO100. When this ligation mixture was transformed into RM120, only about 50% of the ampicillin-resistant colonies expressed galactokinase. By using restriction analysis we were able to show that the plasmids expressing galactokinase were all oriented with the *gyrB* sequences abutting the galactokinase structural gene. These plasmids represented the desired fusion, pGBK1.

It is clear that such a construction also had the very strong λ p_L promoter directing transcription toward the galactokinase gene. To remove p_L, pGBK1 was digested with restriction enzyme *Hpa*I and religated at a low DNA concentration to recircularize the large *Hpa*I fragment. The resulting plasmid, pGBK2, was free of the λ p_L promoter. The entire scheme is outlined in Fig. 1. It should be noted that the *Hpa*I site on pGBK2 was destroyed following the inefficient ligation of the blunt *Hpa*I ends. A more detailed restriction analysis of pGBK2 demonstrated that less than 50 base pairs (bp) was lost as a result of the illegitimate rejoining (data not shown).

Behavior of the fusions. The *gyrB* control sequences were fused to the galactokinase gene to provide a convenient assay for coumermycin-induced expression of the *gyrB* promoter. A mid-log culture of strain RM264 containing the *gyrB-galK* fusion was treated with coumermycin, and the level of galactokinase was measured at various times after this treatment. Figure 2 shows the results of such an experiment. It is readily apparent that coumermycin induced the expression of galactokinase in the *gyrB-galK* fusion above the level of a control untreated culture. However, when the galactokinase gene was downstream from the wild-type galactose operon control sequences it was not induced by coumermycin. Induction by coumermycin is a function of the control sequences upstream of the galactokinase structural gene.

For screening large numbers of strains, induction of galactokinase by coumermycin could be measured without repeating the full time course shown in Fig. 2. Most of the induction took place within the first 1 h following treatment. As a convenient measure of induction we defined "induction ratio" as the level of galactokinase activity in a culture treated with 100 μg of coumermycin A1 per ml at 75 min after treatment divided by the level of galactokinase activity in a parallel untreated culture. Table 2 shows induction data for our fusions and some controls in this format.

From Table 2 it is clear that the upstream p_L promoter (present in the construction pGBK1) contributed little to the expression of the galactokinase gene. Given the strength of this λ promoter, this is a surprising result. It seems likely that a strong terminator sequence must exist upstream of the *gyrB* gene. We have previously described a region of extensive dyad symmetries in the *recF* gene which represents a candidate for such a terminator function (1).

Only a small region immediately upstream of *gyrB* is needed for full promoter function and coumermycin induction. As an initial method for defining the sequences required for coumermycin induction of the *gyrB-galK* fusions, we isolated 200 Tn*9* insertions in plasmid pGBK2 (as described in Materials and Methods). Only five independent insertions which both blocked the expression of *galK* and mapped in the cloned segment were identified. These insertions were located within the 150 bp immediately upstream of the *Sma*I site near the amino terminus of the *gyrB* protein (data not shown).

In an independent experiment we constructed eight deletions within the sequences upstream of *gyrB*. Again we were led to the conclusion that only sequences immediately upstream of *gyrB* are important to promoter function. The single deletion extending to within 50 bp of the *gyrB* initiation codon eliminated promoter function. The remaining

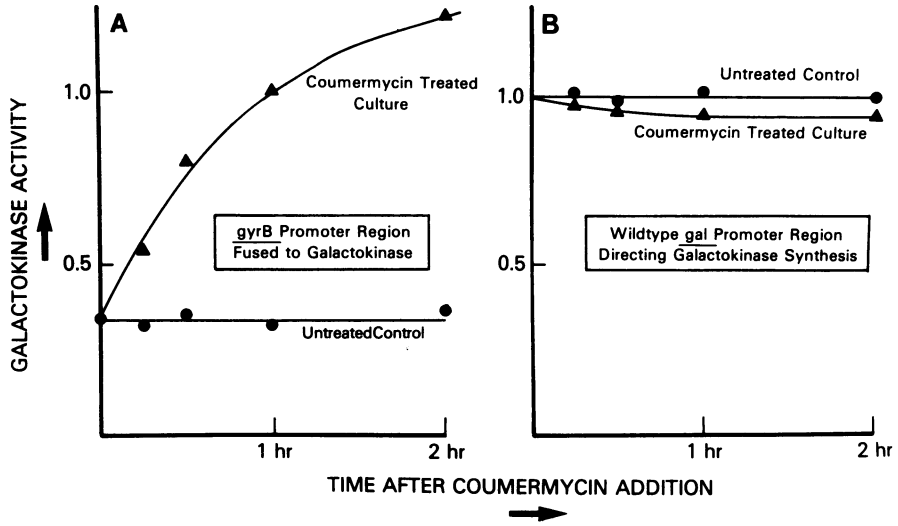

FIG. 2. In vivo induction of galactokinase by coumermycin A1 in *gyrB-galK* construction pGBK2. Cultures of either RM264 (pGBK2) (A) or RM270 (pKG1800) (B) were treated with coumermycin (▲) or were mock treated (●) as described in Materials and Methods. Samples were assayed at the indicated times, and the levels of galactokinase expressed are given as fractions of the activity of RM270 at zero time (see Materials and Methods).

deletions (extending to within 400 bp of the initiating AUG) did not eliminate promoter function and showed normal coumermycin induction (data not shown).

Given the observations described above, we chose to subclone a small *Sau*3A1 fragment which extends from the *Bam*HI site of the vector multilinker insertion to a *Sau*3A1 site 110 bp upstream of the *gyrB* protein start. When this piece was cloned into the *Bam*HI site of vector pKO100, 50% of the resultant clones expressed galactokinase. The orientation of the insertion in these GalK$^+$ clones was the same as that in the original pGBK2 clone. Furthermore, the level of galactokinase expression and the degree of coumermycin induction of one typical subclone, pGBK3, were identical to the values obtained with the parental clone, pGBK2. The identity of the cloned fragment was confirmed by determining the DNA sequence, which is shown in Fig. 3.

The arrow in Fig. 3 indicates the start site for *gyrB* transcription, as previously determined (1). The boxed hexamer has close homology to the -10 promoter consensus sequence. The gyrase-derived fragment in the small subclone spans a region extending from the nucleotides encoding the first 23 amino acids of the *gyrB* protein to a point 110 bp upstream of the protein start. This small region immediately upstream of the *gyrB* gene is evidently all that is needed for expression of the *gyrB* promoter and for its induction by coumermycin treatment.

TABLE 2. Levels of expression and degrees of coumermycin A1 induction of the *gyrB-galK* fusion plasmids and control strains[a]

Strain	Plasmid	Level of expression	Degree of induction
RM267	pKO100	<0.03	NA[b]
RM269	pGBK1	0.31	2.8
RM268	pGBK3	0.32	3.0
RM270	pKG1800	1.00	0.9

[a] The levels of expression are expressed relative to the levels found in strain RM270, which had a wild-type *gal* control region. Assay and induction results were determined and defined as described in the text. Plasmid pGBK3 was derived from PGBK2 as described in the text (plasmids pGBK2 and pGBK3 behaved identically). All values were reproducible within an error of ±10%.

[b] NA, Not applicable.

Cloning and sequence of the *gyrA* regulatory region. In order to study the analogous regulation of *gyrA*, it was necessary to clone the *gyrA* promoter region. A simple approach was to identify a fragment of DNA from the *gyrA* region which caused the expression of the *galK* gene to be inducible by coumermycin. Plasmid pMK90 contains an 11-kb *Bam*HI insertion which encodes the entire *gyrA* gene and its surrounding region (16). This insertion was cleaved from the clone with *Bam*HI and was purified by gel electrophoresis. Once isolated, the 11-kb *Bam*HI fragment was further digested with *Sau*3A1, and the resulting mixture of *Sau*3A1 fragments was cloned into the *Bam*HI site of pKO100. The ligation mixture was transformed into RM120, and a large number of clones expressing galactokinase were identified. Using both the basal level of galactokinase expression and the degree of coumermycin induction as criteria, we were able to classify the collection of clones into the three types shown in Table 3. Further restriction analysis and cloning proved that class I and class II clones contained the same fragment of DNA in opposite orientations (data not shown). Class I clones were inducible by coumermycin and represented candidates for the *gyrA* control region.

The insertion in one such clone, pGAK1, was sequenced as described in Materials and Methods. In the orientation which gave coumermycin induction, there was an open reading frame which extended for 88 amino acid residues to the end of the insertion. The amino terminus of this open reading frame agreed with the amino-terminal sequence of the *gyrA* protein. (We are indebted to Stuart Rudikoff for determining the first 12 amino acid residues of the *gyrA* protein amino terminus [data not shown].) The protein sequence indicates that the terminal methionine was cleaved. Figure 4 shows the sequence of this insertion in the 5′-to-3′ orientation which gave coumermycin-inducible galactokinase expression.

Coumermycin induction is not a rare characteristic of promoters. When growing mid-log *E. coli* cells were pulse-labeled with [^{35}S]methionine after treatment with 100 μg of

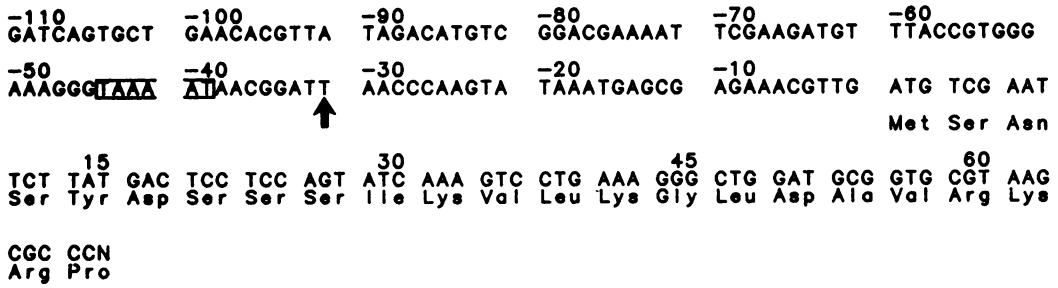

FIG. 3. Sequence of the *Sau*3A1 fragment of inserted *gyrB* material in plasmid pGBK3. The inserted fragment was sequenced as described in Materials and Methods. The start of transcription is indicated by the arrow. The presumptive -10 region is shown by the boxed hexamer. The amino-terminal sequence of the *gyrB* gene as previously determined is given below the DNA sequence. The numbering of the sequence is relative to the start of translation.

coumermycin A1 per ml, 10% of the radioactive protein bands (5 of 50 bands) displayed on a one-dimensional polyacrylamide gel increased in intensity, showing that the rate of synthesis of these proteins had increased (15). In a related experiment Ruth van Bogelen (personal communication) observed that a similar fraction of labeled protein spots on a two-dimensional protein gel were coumermycin inducible. We were interested in using the pKO100 vector system to make a different kind of estimate of the fraction of promoters which are coumermycin inducible.

To do so, we ligated *Sau*3A1-digested *E. coli* chromosomal DNA into *Bam*HI-digested and phosphatase-treated pKO100. The ligation mixture yielded 1,000-fold more ampicillin-resistant transformants than did the control phosphatase-treated vector without the insertion, indicating that essentially every colony contained an insertion. When plated onto MacConkey-galactose agar supplemented with ampicillin, approximately 9% of the colonies showed a Gal^+ phenotype. If we take 256 bp as the average length of a *Sau*3A1 fragment, this value is consistent with the hypothesis that a promoter is present approximately every 2.8 kb. Such a promoter frequency seems reasonable. Plasmid DNA was prepared from 20 Gal^+ transformants picked at random, and the sizes of the insertions were determined by restriction digestion followed by gel electrophoresis. The insertion sizes ranged from 2.5 kb to just over 100 bp; no two clones had the same size insertion, confirming their independent origin (data not shown). This collection of clones was then assayed both for their basal levels of galactokinase expression and for their inducibility by coumermycin (Table 4). Some clones were induced, some clones were inhibited, and some were relatively unaffected. Clearly, induction of gene expression by coumermycin treatment is not a unique property of the gyrase genes but is a characteristic shared by many promoters.

TABLE 3. Levels of expression and degrees of coumermycin A1 induction in the three classes of plasmids obtained from the collection of clones derived from the *Sau*3A1 fragments of the 11-kb *Bam*HI *gyrA* insertion of pMK90[a]

Plasmid class or strain	Plasmid	Level of expression	Degree of induction
Plasmid classes			
I	pGAK1 (*gyrA*)	0.54	2.3
II	pCA2 (inverse *gyrA*)	0.40	1.2
III	pCA3 (other)	0.14	1.4
Control strains			
RM267	pKO100	<0.03	NA[b]
RM268	pGBK3 (*gyrB*)	0.31	3.2
RM270	pKG1800 (*gal*)	1.00	1.0

[a] Assay and induction results were determined and defined as described in the text and in the legend to Fig. 2. All values were reproducible within an error of ±10%.

[b] NA, Not applicable.

DISCUSSION

In previous work, we showed that the chromosomal *gyrB* transcript is induced by coumermycin, indicating that the DNA relaxation-dependent stimulation of GyrB protein synthesis is controlled at the transcriptional level (1). The present work confirmed and extended this result. Plasmid pKO100, into which we inserted the *gyrB* and *gyrA* upstream regions, is a fusion vector with stop codons in all three reading frames, which guarantee that the resulting fusions place galactokinase under transcriptional control of the fused upstream sequences (14). The coumermycin-induced galactokinase synthesis shown by our fusion plasmids must result from increased transcription originating in the fused upstream gyrase sequences. These fusions demonstrated a two- to threefold accumulated increase in the total level of galactokinase activity following 75 min of exposure to coumermycin A1. Such an increase in the level of galactokinase activity is roughly consistent with the approximately 10-fold increase in the rate of synthesis reported for gyrase expression following relaxation of the template DNA (15), when integrated over the 30 to 40% increase in cell mass observed in these experiments.

By successively removing more and more of the sequence upstream of *gyrB* and then finally recloning a small 160-bp fragment, we demonstrated that only a small region is important to the expression of *gyrB* and its induction by coumermycin treatment. As with most other transcriptional control mechanisms, relaxation-stimulated transcription (as inferred by coumermycin induction) appears to be a property of a short sequence of DNA.

The coumermycin induction phenotype has allowed us to identify a fragment of DNA which contains the amino terminus of the *gyrA* protein and the associated upstream sequences which direct the expression of the *gyrA* gene. The particular fragment of DNA which contains the *gyrA* promoter also shows promoter activity when it is inverted. We note that there is an open reading frame beginning with an AUG which reads to the end of the fragment in the direction opposite the *gyrA* reading frame. Upstream of this other reading frame are sequences which show reasonable homologies to a ribosome binding site and a -10 promoter sequence (these features are not identified in the legend to Fig. 4). The

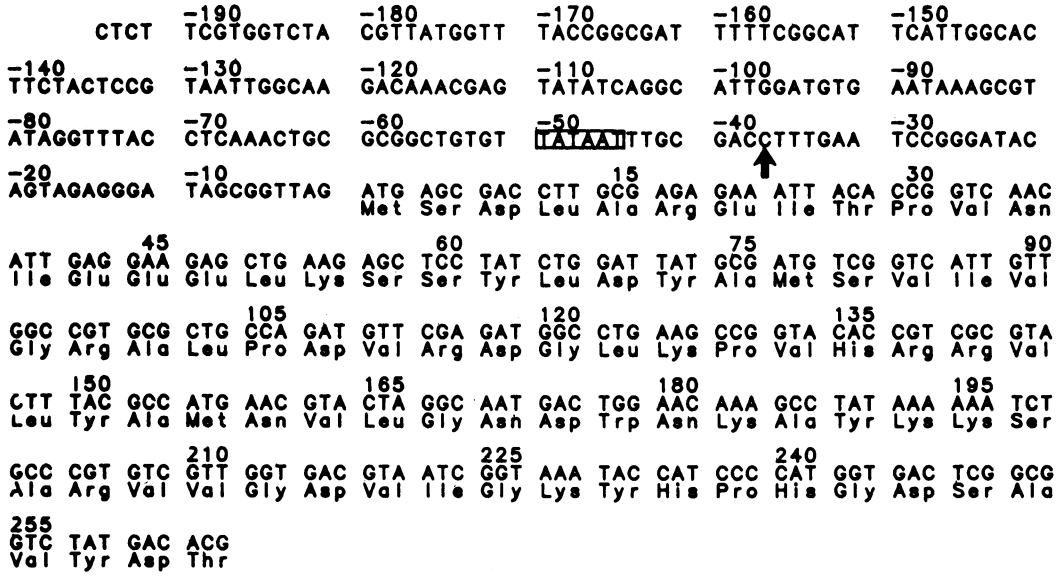

FIG. 4. Sequence of the *Sau*3A1 fragment inserted in plasmid pGAK1. The inserted fragment was sequenced as described in Materials and Methods. The arrow is centered in the region where transcription starts (Steve Swanberg, personal communication). The presumptive -10 region is shown by the boxed hexamer. The amino-terminal sequence of the *gyrA* gene as determined for this paper is given below the DNA sequence. The numbering of the sequence is relative to the start of translation.

transcription divergent from *gyrA* is not strongly coumermycin inducible. Therefore, we conclude that coumermycin induction is associated with a particular transcript and is not a general property of a DNA fragment.

With the sequences for both the *gyrA* and *gyrB* control regions in hand, it is interesting to look for similarities and homologies. Both genes have promoters which are located similar short distances upstream from the initiation codon (38 bp in the case of *gyrB* and 44 bp in the case of *gyrA*). For the *gyrB* promoter we have S1 transcript mapping data (1) which confirm that the boxed -10 region in Fig. 3 is in fact the promoter. Analysis of the *gyrA* sequence by the promoter search algorithm of Mulligan et al. (17) identified the boxed -10 region as belonging to the only strong promoter on the *gyrA* fragment (data not shown). Both of these presumptive promoters have excellent homologies to the consensus -10 region but rather poor homology to the consensus -35 region. In addition, both transcripts appear to start with a pyrimidine nucleotide, which is relatively rare among *E. coli* promoters.

TABLE 4. Levels of expression and degrees of coumermycin A1 induction of the random *E. coli Sau*3A1 clones[a]

Strain	Level of expression	Degree of induction
RM350	0.40	1.47
RM351	0.36	0.71
RM352	0.33	1.04
RM353	0.39	0.86
RM354	0.56	1.49
RM355	0.23	1.10
RM356	0.13	0.58
RM357	0.57	0.94
RM358	0.34	0.98
RM359	0.09	4.05
RM360	0.05	0.27
RM361	0.16	0.89
RM362	0.48	0.98
RM363	0.15	0.68
RM364	0.40	1.42
RM365	0.11	3.61
RM366	0.08	6.11
RM367	0.19	0.90
RM368	0.16	2.30
RM369	0.17	1.29

[a] Assay and induction results were determined and defined as described in the text and in the legend to Fig. 2. All results except those for strains RM360 and RM366 were reproducible within an error of ±10%. The values for strains RM360 and RM366 were reproducible at a level of ±50%

The most striking similarity between the promoters is the homology shown in Fig. 5. That GTTTACC appears in two relatively short sequences is improbable. The further constraint of its placement in similar locations (just upstream of the -10 region) and the additional homology on its upstream flank make its accidental occurrence even more unlikely. We also note that GGTTTACC is found a second time in the *gyrA* sequence at position -170. The functional significance of this sequence is unclear, however. It is not required for relaxation-stimulated transcription; in other work we have shown that it can be deleted from both *gyrA* and *gyrB* control regions without affecting coumermycin induction (submitted for publication). The significance of this and other similari-

FIG. 5. Comparison of the *gyrA* and *gyrB* control regions directing transcription in the *gyr-galK* fusions. Homology is found in the sequences 5′ to the -10 region in both *gyrA* and *gyrB*. Sequences are aligned to show homology (asterisks) and are numbered as in Fig. 3 and 4.

ties between the *gyrA* and *gyrB* promoters demands further study.

We have previously shown that coumermycin induces DNA gyrase synthesis by inhibiting the actions of the enzyme; there is no induction in coumermycin-resistant *gyrB* mutants, and, on the other hand, a temperature-sensitive *gyrB* mutant induces gyrase synthesis at a high temperature (15). In the mutant, pGBK3-directed galactokinase synthesis is induced at the high temperature (unpublished data). The inhibition of gyrase in coumermycin-treated cells has been shown to lead to relaxation of chromosomal, plasmid, and phage DNAs (7, 11). We found that relaxation of the DNA template in a cell-free protein-synthesizing system was sufficient to induce gyrase synthesis (15).

In addition to this rather simple effect, coumermycin treatment of cells affects more complex regulatory networks. A transient induction of a group of proteins also induced by heat shock has been noted (15, 23). This induction does not occur in an *rpoH* mutant in which heat shock is blocked (26), but gyrase induction is normal (unpublished data).

There is also delayed induction of *recA* protein synthesis by coumermycin (15, 21). In a *lexA3* mutant which blocks induction of the SOS system (3), this effect is not seen, but once again induction of gyrase occurs normally (unpublished data).

Treatment of cells with coumermycin and the ensuing DNA relaxation have a multiplicity of effects which together influence a large fraction of *E. coli* genes. Immediate and sustained increases or decreases in transcription like those of the gyrase genes each apply to 10 to 20% of the promoters; the transient response mediated by the heat shock system affects approximately 20 genes; and the delayed SOS response probably affects other genes besides *recA*. The recent demonstration that well-characterized *hisW* mutations, which have pleiotropic regulatory consequences during steady-state growth, are alleles of *gyrA* shows that DNA gyrase plays a role in regulating gene expression during normal cell growth (K. Rudd and R. Menzel, Proc. Natl. Acad. Sci. USA, in press). DNA supercoiling is evidently a significant regulatory factor in enteric bacteria. The demonstration that expression of *topA*, the gene for topoisomerase I, is decreased by a reduction in the level of DNA supercoiling (24) reinforces our previous conclusion, based on the opposite regulation of gyrase, that DNA supercoiling is homeostatically controlled.

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