Gyrase Inhibitors Can Increase gyrA Expression and DNA Supercoiling

ROBERT J. FRANCO† AND KARL DRLICA1,2*

Public Health Research Institute, 455 First Avenue,1 and Department of Microbiology, New York University School of Medicine,2 New York, New York 10016

Received 25 July 1989/Accepted 19 September 1989

Treatment of bacterial cells with inhibitors of gyrase at high concentration leads to relaxation of DNA supercoils, presumably through interference with the supercoiling activity of gyrase. Under certain conditions, however, the inhibitors can also increase supercoiling. In the case of coumermycin A₁, this increase occurs at low drug concentrations. Oxolinic acid increases supercoiling in a partially resistant mutant. We found that increases in chromosomal DNA supercoiling, which were blocked by treatment with chloramphenicol, were accompanied by an increased expression rate of gyrA. This result is consistent with gyrase being responsible for the increase in supercoiling. In wild-type cells, increases in gyrA expression were transient, suggesting that when supercoiling reaches sufficiently high levels, gyrase expression declines. Oxolinic acid studies carried out with a ΔtopA strain showed that drug treatment also increased plasmid supercoiling. The levels of supercoiling and topoisomer heterogeneity were much higher when the plasmid contained one of several promoters fused to galK. Since oxolinic acid causes an increase in gyrA expression, it appears that gyrase levels may be important in transcription-mediated changes in supercoiling even when topoisomerase I is absent.

It is becoming increasingly evident that certain environmental conditions can influence DNA supercoiling (1, 5, 16). Since supercoiling is an important factor in the interaction of many proteins with DNA, it may be that the changes in supercoiling are important for the adaptation of bacteria to changes in growth environment. In Escherichia coli, DNA supercoiling is controlled largely by two enzymes, DNA gyrase (13) and DNA topoisomerase I (44). Gyrase introduces negative supercoils into DNA in vitro, and inhibition of the enzyme in vivo blocks supercoiling of bacteriophage lambda DNA during superinfection of a lysogen (11, 12) and leads to relaxation of supercoils in the bacterial chromosome (7, 23, 37). Topoisomerase I appears to modulate the effect of gyrase by providing a relaxing activity; mutations in topA, the gene encoding topoisomerase I (38, 41), can lead to higher than normal levels of supercoiling (4, 34, 35). Superciling itself can influence expression of the genes encoding gyrase and topoisomerase I in a way thought to result in the homeostatic regulation of supercoiling (25, 42, 43). Even the small changes in supercoiling expected to arise from changes in temperature or intercalating dyes appear to be corrected by corresponding changes in linking difference (8, 15). Thus, supercoiling levels are controlled; how they are altered by environmental changes is not known.

The physiological effects of altering DNA supercoiling can be studied by perturbing the balance of gyrase and topoisomerase I. Studies of this type have usually involved lowering negative supercoiling; little is known about effects associated with increasing supercoiling. The ability to increase supercoiling may be quite important in the regulation of gene expression, since anaerobiosis and high osmolarity, the two best-studied environmental factors that alter supercoiling, both raise supercoiling.

Levels of negative supercoiling can be raised above normal in several ways. Mutation of topA is one (4, 34). Under special circumstances, supercoiling can also be increased by inhibitors of gyrase. Oxolinic acid, an inhibitor of the A subunit of gyrase (11, 40), increases supercoiling in a mutant strain partially resistant to the drug (23, 33). In wild-type cells, supercoiling can be raised by treatment with low concentrations of coumermycin A₁ (23), an inhibitor of the B subunit of gyrase (12). Transcription can also stimulate increases in negative supercoiling, a phenomenon that to date has been observed only in plasmids (6, 8a, 32, 45).

Inhibitors that shift supercoiling to elevated levels provide a way to study the behavior of topoisomerase genes and the control of supercoiling. For example, inhibitors have been used to show that expression of topA increases when supercoiling becomes high (43). This response by topA would counter perturbations that tend to raise supercoiling. In this study, we report that the rate of expression of gyrA also increases when inhibitors raise supercoiling, consistent with gyrase being responsible for the increase in supercoiling. But the increase in gyrA expression is transient in wild-type cells, which suggests that when supercoiling becomes sufficiently high, gyrase expression decreases.

MATERIALS AND METHODS

Bacterial strains, plasmids, and culture conditions. The bacterial strains used (Table 1) are derivatives of E. coli K-12. Strain GP200 is a Nal\(^{r}\) transductant of DM800, a ΔtopA mutant containing a compensating gyrB mutation. Strain GP200 has the unusual properties of being only partially resistant to nalidixic and oxolinic acids and of increasing its chromosomal DNA supercoiling when exposed to oxolinic acid (23, 33). These properties appear to be due to the topoisomerase mutations present, since they can be transferred to other strains by transduction (33). We have assigned the Nal\(^{r}\) allele to gyrA on the basis of cotransduction of this allele and temperature resistance into the gyrA43(Ts) strain KD103 (a spontaneous glpT, phosphomycin-resistant [Phospho\(^{r}\)] derivative of strain KNK453 [19]; phosphomycin resistance is 60% cotransducible with gyrA). Two-thirds of the Nal\(^{r}\) temperature-resistant transductants
from GP200 into KD103 were also Phospho, the result expected if the Nal' allele maps in gyrA (9, 18). Transduction of the Nal' allele from GP200 into a wild-type strain results in full resistance to nalidixic acid, indicating that this gyrA allele behaves abnormally when topA is deleted (33). Since transduction of topA into DM800 raises levels of gyrase expression (10), it is likely that GP200 has a low level of gyrase; this may account for lowered resistance to nalidixic acid.

Plasmids that have galactokinase (galK) expression under the control of either the gyrA (pGAK1), gyrB (pGBK3), or galK (pKG1800) promoter or no promoter (pKO100) were gifts from Rolf Menzel (see reference 27 for constructions). These plasmids are derivatives of pBR322 in which tet has been replaced by a multicloning site and galK (24) such that transcription of galK occurs in the same orientation as tet, which in pBR322 is opposite to that of bla.

Liquid cultures were grown at 37°C in glucose M9 minimal salts medium (28). For measurement of GyrA and GyrB synthesis rates, the medium was supplemented as described below. For DNA supercoiling assays, cells were grown in glucose M9 salts minimal medium supplemented with Casamino Acids (4.6 mg/ml; Difco Laboratories, Detroit, Mich.), cysteine (56 μg/ml), and thiamine (0.4 μg/ml).

Chemicals and reagents. Oxolinic acid was a gift from Warner-Lambert Research Institute and was stored at a concentration of 400 μg/ml in 100% dimethyl sulfoxide (DMSO). Coumermycin A1 was a gift from Bristol Laboratories, Syracuse, N.Y. A fresh stock solution of coumermycin (5 mg/ml in dimethyl sulfoxide) was made weekly and stored at 4°C. Chloramphenicol, pyruvate kinase, catalase, and chloroquine (diphosphate salt) were products of Sigma Chemical Co., St. Louis, Mo. Chloramphenicol was freshly prepared for each experiment at a concentration of 100 mg/ml in 95% ethanol. Amphotolyses (Bio-Lyte 5-7 and 3-10) and agarose (ultrapure DNA grade) were purchased from Bio-Rad Laboratories, Richmond, Calif. [methyl-3H]thymidine (40 to 70 Ci/mmol) and d-[1-14C]galactose (60 mCi/mmol) were purchased from Dupont, NEN Research Products, Boston, Mass. [4,5-3H]leucine (124 Ci/mmol) and l-[35S]methionine (1,210 Ci/mmol) were purchased from Amersham Corp., Arlington Heights, Ill. Purified subunits A and B of DNA gyrase were obtained from M. Gellert, National Institutes of Health, Bethesda, Md.

Measurement of synthesis rates of gyrases. Synthesis rates of the A and B subunits of gyrases were determined by measuring the incorporation of radioactive methionine into cellular proteins. Cells were first grown in M9 medium lacking methionine and leucine but containing the other 18 amino acids, each at a concentration of 40 μg/ml, and biotin at 1 μg/ml. They were then labeled by addition of [3H]leucine (50 μCi/ml) and grown for approximately two generations. Excess unlabeled leucine (300 μg/ml) was added for 5 min before the addition of either oxolinic acid or coumermycin. After addition of drug, cells were pulse-labeled by addition of [35S]methionine (20 μCi/ml) for 1 min, followed by addition of unlabeled methionine (300 μg/ml) for 2 min to allow completion of synthesis of labeled proteins. Cells were then chilled on ice.

Radioactively labeled cells were collected by centrifugation and processed as described by O'Farrell (29), with the following modifications. Cells were suspended in 100 μl of lysis buffer (9 M urea, 0.2% Nonidet P-40, 1.6% amphotolysate pH 5 to 7, 0.4% amphotolysate pH 3 to 10, 5% 2-mercaptoethanol) and lysed by three freeze-thaw cycles. The first-dimension isoelectric focusing gels were electrophoresed at 400 V for 6,000 to 10,000 V-h. The gels were shaken in 5 ml of 10% glycerol-2.3% sodium dodecyl sulfate-5% 2-mercaptoethanol—62.8 mM Tris hydrochloride (pH 6.8) for 1 h and loaded onto sodium dodecyl sulfate-containing polyacrylamide gels. After electrophoresis, gels were fixed in 50% methanol-12% acetic acid for at least 1 h, and they were then soaked for several hours in 10% ethanol-5% acetic acid. Gels were then either silver stained (14) or prepared for autoradiography by drying on 3MM paper (Whatman, Inc., Clifton, N.J.) under vacuum and heat.

Protein spots were excised from dried gels and incubated in 500 μl of hydrogen peroxide at 70°C for 5 h. Catalase (400 μl of a 250-μg/ml solution) was added, and incubation was continued for 15 min. Radioactivity was then measured after the samples were shaken in 19 ml of Liquiscint scintillation fluid for 24 h at room temperature. The rate of synthesis of a given protein was taken as the 35S/3H ratio in the excised spot (3H label corrects for differences in protein recovery from gels). Quench curves were determined for each experiment to correct for differences in counting efficiency among samples. Specific activities from different regions of single spots were identical, reducing the possibility that neighboring spots influenced the determinations.

Measurement of DNA supercoiling. The effects of oxolinic acid and coumermycin on average chromosomal DNA supercoiling were assessed by determining the concentration of ethidium bromide necessary to titrate the negative supercoils present in nucleoids extracted from drug-treated cells. The cell lysis procedure is a modification of that of Stonington and Pettijohn (39); titration of supercoils was measured by sedimentation of [3H]-labeled nucleoids into sucrose density gradients. Both procedures have been described previously (7) but were modified in the following ways: solution C contained 0.2% Sarkosyl (CIBA-GEIGY Corp., Summit, N.J.) in addition to the other detergents, and cell lysates were loaded directly onto the sucrose gradients.

Plasmid supercoiling was compared by gel electrophoresis in agarose containing chloroquine at concentrations indicated in the figure legends. Plasmid DNA was isolated by the method of Holmes and Quigley (17), followed by centrifugation in CsCl.

RESULTS

Correlation of increased chromosomal supercoiling with increased gyrA expression. In principle, elevated levels of supercoiling could arise from an increase in gyrase, from a decrease in topoisomerase I, or both. Increased supercoiling generated by oxolinic acid occurs only in strains carrying a
topA mutation (33); therefore, in this case changes in topoisomerase I levels cannot be involved. To examine the possibility that gyrase increases supercoiling, we measured the rate of synthesis of gyra after oxolinic acid treatment of strain GP200, a ΔtopA mutant partially resistant to oxolinic acid (see Materials and Methods for strain description). Cells were pulse-labeled with [35S]methionine, and cell extracts were subjected to two-dimensional gel electrophoresis (29) (Fig. 1). The Gyra protein spot, identified as described in the legend to Fig. 1, was excised from gels, and the amount of 35S incorporated into the protein was determined. Since gyrase is a stable protein (25), determining the amount of 35S incorporated into the Gyra protein during a short time should reliably estimate the rate of Gyra synthesis. Oxolinic acid increased the rate of synthesis of Gyra by twofold in GP200 (Fig. 2A) under conditions previously shown to increase average levels of chromosomal DNA supercoiling by about 50% (23, 33). The gyrB gene is mutant in strain GP200, and GyrB does not migrate to the same position as does the wild-type protein during electrophoresis; therefore, we could not unambiguously measure the effect of oxolinic acid on GyrB synthesis by using this system.

Since supercoiling can also be increased by treatment of wild-type cells with low concentrations of coumermycin (23), rates of Gyra synthesis were measured under these inhibitor conditions. Treatment of strain DM4100 with 1 μg of coumermycin per ml produced a transient, threefold stimulation of Gyra production, with maximal stimulation occurring 20 min after addition of the drug (Fig. 2B). After treatment with coumermycin and separation of proteins by electrophoresis, the protein spot corresponding to GyrB disappeared. A more intense protein spot having the same molecular weight appeared which behaved as though it were more acidic than GyrB. Although we cannot say unequivocally that this new spot is GyrB, it is reasonable to assume that GyrB production is stimulated by coumermycin, since it has been shown by other assays that synthesis of both subunits of gyrase is stimulated to similar degrees by higher concentrations of this drug (25).

Although high concentrations of the inhibitors also stimulate gyrase expression, supercoiling does not increase. This was first seen with coumermycin (25) under conditions that relax chromosomal DNA (7). An intermediate effect could be seen after oxolinic acid treatment of wild-type cells. At oxolinic acid concentrations that maximally inhibit DNA synthesis, little change in supercoiling occurs (23, 36; data not shown), and the stimulation of Gyra and gyrB expression is transient (Fig. 2B). Therefore, inhibitors of gyrase increase rates of gyrase expression, but the degree and direction of supercoiling change probably depend on whether the drug concentration is high enough to block the supercoiling activity of gyrase and on the response of topoisomerase I, if present, to the perturbation.

Blockage by chloramphenicol of increases in supercoiling induced by gyrase inhibitors. If increased synthesis of gyrase
FIG. 3. Effect of oxolinic acid and chloramphenicol on chromosomal DNA supercoiling in GP200. Exponentially growing cultures of GP200 labeled with [3H]thymidine (10 μCi/ml for one generation) were treated with chloramphenicol (200 μg/ml), oxolinic acid (5 μg/ml), or both. Cells were lysed, and nucleoid sedimentation rates were determined at various concentrations of ethidium bromide. For each gradient, the sedimentation rate of the nucleoids was determined relative to that of a 14C-labeled bacteriophage T4B marker (1025 S [3]), using the means of the radioactivity peaks. (A) Untreated (○) and chloramphenicol treated (●); (B) treated with oxolinic acid (○) and chloramphenicol plus oxolinic acid (●). The dashed line marks the sedimentation minimum of nucleoids from untreated wild-type cells.

is responsible for the oxolinic acid-induced increase in supercoiling, then the increase in supercoiling should be blocked by the inhibition of protein synthesis. Chloramphenicol, at a concentration sufficient to inhibit protein synthesis by more than 95%, blocked the ability of oxolinic acid to increase supercoiling in strain GP200 (Fig. 3). In these measurements, nucleoids were sedimented into sucrose density gradients containing various concentrations of ethidium bromide. Under the conditions used, the ethidium bromide concentration at the sedimentation minimum is proportional to the superhelicity density [2]. Since chloramphenicol does not affect the ability of oxolinic acid to rapidly inhibit DNA synthesis in GP200 (data not shown), it is unlikely that chloramphenicol blocks the increase in supercoiling by preventing cellular uptake of oxolinic acid. Chloramphenicol also blocked the ability of low concentrations of coumermycin (1 or 3 μg/ml) to increase supercoiling (data not shown).

FIG. 4. Kinetics of increase in supercoiling induced by inhibitors of gyrase. (A) [3H]labeled cultures of strain GP200 were treated with oxolinic acid (5 μg/ml) for the indicated times and then rapidly chilled. Cells were lysed, and sedimentation rates of the nucleoids were determined at various concentrations of ethidium bromide as described in the legend to Fig. 3. The relative superhelicity density of the DNA, expressed as the ethidium concentration at the sedimentation minimum, was determined from plots similar to those shown in Fig. 3. (B) Cultures of strain DM4100 were treated with coumermycin (1 μg/ml) for the indicated times, and the relative superhelicity density of the nucleoids was determined as described for panel A.

Increase in gyrase expression without detectable DNA relaxation. Expression of the gyrase genes in vitro is higher when DNA is relaxed than when it is supercoiled [25]. Therefore, it is possible that the inhibitor treatments described above cause a decrease in supercoiling that then stimulates gyrase expression and subsequently increases supercoiling. We were unable to detect transient relaxation at the whole-chromosome level when strain GP200 was treated with oxolinic acid (Fig. 4A) or when strain DM4100 (wild type) was treated with low concentrations of coumermycin (Fig. 4B). In these experiments, drug incubations were stopped by rapidly chilling the cultures. Since this procedure blocks gyrase activity [37], it is unlikely that the time (10 min) needed to harvest and lyse the cells contributed significantly to incubation times. Rapid (completed within a few minutes), slight (less than 10%), or local relaxation could have escaped detection by the assay used.

Increase in plasmid supercoiling by oxolinic acid treatment. To determine whether the oxolinic acid-induced increase in supercoiling is a general property of bacterial replicons in strain GP200, we also examined supercoiling of plasmids. To minimize the topological effects that might be generated by transcription from divergent promoters [20, 32], plasmid pKO100 was examined. In this derivative of pBR322, a promoterless galK gene has replaced tet. Plasmid DNA was electrophoresed in the presence of chloroquine under conditions in which the more negatively supercoiled topoisoforms migrate more rapidly. Oxolinic acid caused plasmid
DNA supercoiling to increase (Fig. 5, lanes 1 and 2), a result consistent with studies on chromosomal DNA (23).

In previous work, it had been discovered that transcription of the tet gene of pBR322 causes a large increase in negative supercoiling in the ΔtopA strain DM800 (32). Li and Wang (20) subsequently proposed that tracking of RNA polymerase along DNA could establish two topological domains. Negative supercoils would be generated behind the transcription complex, and positive supercoils would be generated in front of it. The negative and positive supercoils would be relaxed by topoisomerase I and gyrase, respectively. As observed, loss of topoisomerase I allows the accumulation of negative supercoils (32), and inhibition of gyrase allows the accumulation of positive supercoils (21, 45). An increase in gyrase expression also correlated with a transcription-dependent increase in negative supercoiling: oxolinic acid caused a much greater increase in plasmid supercoiling in transformants of strain GP200 if the gal promoter had been fused to galK (Fig. 5; compare plasmids pKO100 [lane 1] and pKG1800 [lanes 7]). In the absence of the drug, the two plasmids exhibited little difference (Fig. 5, lanes 2 and 8).

The topoisomers of pKG1800 exhibited extensive linking-number heterogeneity after oxolinic acid treatment of strain GP200-4 (Fig. 6A), as had been seen previously with transcription-stimulated increases in supercoiling of pBR322 in strain DM800 (32). In this type of experiment, electrophoresis was first carried out at a moderately high concentration of chloroquine to separate the plasmid topoisomers into a series of bands. The less negatively supercoiled species became positively supercoiled at this chloroquine concentration; the more negatively supercoiled species remained negatively supercoiled. A second dimension of electrophoresis was then carried out at a higher chloroquine concentration to separate species that had remained negatively supercoiled (left arc) in the first dimension from those that were positively supercoiled (right arc). With pKO100 from strain GP200-1, extensive heterogeneity was seen only when gels were overloaded (Fig. 6B).

We also examined plasmid supercoiling in derivatives of pKO100 in which the gyrA and gyrB promoters were fused to the coding region of galK. Flanking DNA extended from -194 to +264 for gyrA and from -110 to +69 for gyrB (+1 is the start of transcription) (27). Oxolinic acid increased the negative supercoiling and the topoisomer heterogeneity of these plasmids in a manner similar to that observed when the galK promoter was present (Fig. 5 and 6). Therefore, the gyrase promoters were sufficiently active for transcription-dependent increases in supercoiling to occur in strains GP200-2 and GP200-3. Using the method of McKenney et al. (24), we then measured galactokinase production to assess the effect of oxolinic acid and the very large increase in negative supercoiling on the activity of the gyrase promoters. The level of galactokinase detected in cells containing plasmids with the gyrA, gyrB, or galK promoter fused to galK (strain GP200-2, GP200-3, or GP200-4, respectively) was about 27 times that of the control (strain GP200-1 containing pKO100), and the addition of oxolinic acid reduced it by only 10 to 25% for each promoter (data not shown). Therefore, the promoters were relatively insensitive to the very high levels of negative supercoiling in these plasmids.

**DISCUSSION**

Inhibitors of gyrase generally relax bacterial DNA (7, 23); however, in special circumstances they can also increase
supercoiling in chromosomes (23, 33; Fig. 3 and 4) and in plasmids (Fig. 5 and 6). Increased gyrA expression (Fig. 2) accompanies inhibitor-related increases in supercoiling, suggesting that increased gyrase expression accounts for the increase in supercoiling. As expected, chloramphenicol, an inhibitor of protein synthesis, blocks the increase in supercoiling caused by the two inhibitors of gyrase (Fig. 3 and data not shown).

How gyrA expression is increased by inhibitors is not well understood. In vitro transcription from gyrA and gyrB is much higher from relaxed templates (25), with the gyrA and gyrB promoters being the supercoiling-sensitive regions of the genes (26). However, both oxolinic acid and coumermycin can also increase gyrA expression under conditions in which supercoiling increases (Fig. 2 and 4). One testable idea is that the SOS response is somehow involved, since both drugs induce that response. It could also be argued that a transient relaxation occurred that was too rapid or too small (less than 10%) for us to detect. That view would be consistent with the observations obtained with extensive DNA relaxation (25–27). Also consistent is the possibility that a local relaxation occurs around the gyrA gene, one that would have escaped detection by our whole-chromosome measurements. Such a phenomenon, if peculiar to the chromosome, might help explain why oxolinic acid increases gyrA expression from the chromosome in GP200 (Fig. 2A) but decreases expression slightly when the promoter is fused to galK in plasmid pGAK1 (data not shown). Indeed, the gyrA genes might be good regions of the chromosome to explore for local changes in supercoiling.

The transient nature of the increase in gyrase expression (Fig. 2B) fits with the hypothesis that supercoiling is homeostatically regulated. According to that model (25), decreased supercoiling (7) causes expression of gyrase to increase (25) and expression of topoisomerase I to decrease (42). This would tend to bring supercoiling back to normal levels, which would in turn reduce gyrase expression and increase topoisomerase I expression. Raising supercoiling above normal levels should have the opposite effect. Topoisomerase I promoters, cloned onto a plasmid, do increase their activity in strain GP200 after oxolinic acid treatment (43), and in wild-type cells the rate of gyrA expression declines after the initial increase generated by coumermycin (Fig. 2B).

A transient increase in expression is also seen after oxolinic acid treatment of wild-type cells (Fig. 2B), but so far we have not observed changes in average chromosomal supercoiling under these conditions. Transitory or local changes in supercoiling could have escaped detection. When both gyrases are mutant and topA is deleted (strain GP200), an aspect of control appears to be lost: during oxolinic acid treatment of strain GP200, the rate of gyrA expression failed to drop once chromosomal supercoiling became elevated (Fig. 2A).

Plasmid supercoiling increases much more dramatically after oxolinic acid treatment of a partially resistant mutant (GP200) if the galK gene in the plasmid is fused to a promoter (Fig. 5 and 6). Promoter-dependent increases in negative supercoiling had been reported previously for the tet gene in pBR322 in strain DM800 (32), from which GP200 was derived. In the case of DM800, rifampin treatment eliminates the high level of supercoiling (6), and it is likely that both DM800 and GP200 the increase in supercoiling is stimulated by transcription. Our experiments with strain GP200 extend the previous studies in two ways. First, transcription-stimulated increases in supercoiling in topA mutants can occur when galK is substituted for tet (during the course of this work, a similar observation was made by Wu et al. [45]; for discussion of differences between tet and gal, see reference 22). This finding supports the idea that transcription-mediated changes in supercoiling are common to many genes. Second, the absence of topoisomerase I is not a sufficient condition for transcription to increase negative supercoiling; strain GP200 contains a deletion of topA, but plasmids do not exhibit the high degree of supercoiling without oxolinic acid treatment (Fig. 5 and 6). In strain DM800, oxolinic acid treatment was not required to observe the very high level of supercoiling (31, 32). We speculate that in strain GP200 the gyrA307 allele lowers gyrase activity and diminishes the effect of transcription on supercoiling in plasmids (Fig. 5). Oxolinic acid stimulates production of gyrA and presumably gyrase (Fig. 2); then very high levels of supercoiling with considerable topoisomerase heterogeneity are observed in appropriate plasmids (Fig. 5 and 6). Thus, it appears that the imbalance between gyrase and topoisomerase I must exceed a threshold value before the high levels of supercoiling are observed. Since transcription itself seems to be able to create topoisomerase imbalances in wild-type bacteria (8a) and in yeast cells (30), it is likely that transcription-mediated alteration of DNA supercoiling is a general phenomenon. Factors important in transcription-mediated supercoiling, other than topoisomerase imbalance, include the orientation of transcription units (20, 45), the level of transcription (8a), and anchorage of transcription units (22). We now need to determine whether transcription is an important factor in determining levels of chromosomal DNA supercoiling.

ACKNOWLEDGMENTS

We thank Martin Gellert for providing the A and B subunits of gyrase, Rolf Menzel for providing plasmids, and James Wang for communicating results prior to publication. We also thank the following for critical comments on the manuscript: Richard Burger, Marla Gennaro, John Kornblum, Rolf Menzel, Ellen Murphy, and James Wang.

This work was supported by Public Health Service grants from the National Institutes of Health, grant NP565 from the American Cancer Society, and grant PMB 8718115 from the National Science Foundation.

LITERATURE CITED

INHIBITORS OF GYRASE