Cell, Vol. 53, 433-440, May 6, 1988, Copyright © 1988 by Cell Press

Transcription Generates Positively and Negatively Supercoiled Domains in the Template

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Summary

We show that transcription of a DNA molecule inside a bacterium is accompanied by local and temporal supercoiling of the DNA template: as transcription proceeds, DNA in front of the transcription ensemble becomes positively supercoiled, and DNA behind the ensemble becomes negatively supercoiled. Because bacterial gyrase and topoisomerase I act differently on positively and negatively supercoiled DNA, the formation of twin supercoiled domains during transcription is manifested by a large increase or decrease in the linking number of an intracellular plasmid when bacterial DNA gyrase or topoisomerase I, respectively, is inhibited. Such changes in linking number are strongly dependent on transcription of the plasmid in *cis* and on the relative orientations of transcription units on the plasmid. These results indicate that the state of supercoiling of bacterial DNA is strongly modulated by transcription, and that DNA topoisomerases are normally involved in the elongation step of transcription.

Introduction

The state of supercoiling of intracellular DNA in bacteria is controlled by at least two opposing topoisomerases, DNA topoisomerase I and gyrase (DNA topoisomerase II). In Escherichia coli the two enzymes appear to be homeostatically regulated: a decrease in the degree of negative supercoiling elevates the transcription of the *gyrA* and *gyrB* genes, encoding the two subunits of gyrase, and reduces the transcription of the *topA* gene, encoding DNA topoisomerase I; an increase in the degree of negative supercoiling has the opposite effects on the expression of these genes (Menzel and Gellert, 1983; Tse-Dinh, 1985; Tse-Dinh and Beran, 1988). E. coli *topA* mutants are not viable unless they acquire compensatory mutations, some of which reduce the level of gyrase (DiNardo et al., 1982; Pruss et al., 1982; Raji et al., 1985). The suppression of the lethal phenotype of *topA* mutations by compensatory mutations in the *gyrA* and *gyrB* genes supports the view that the proper level of supercoiling of bacterial DNA in E. coli is essential and is controlled by the diametric actions of the two enzymes.

Results of linking-number measurements of intracellular DNAs in topoisomerase mutants are generally in agreement with the above view (reviewed in Drlica, 1984; Vosberg, 1985; Wang, 1985, 1987; see also Giaever et al., 1988). Two recent observations, however, appear to be unexplained by this simple model. First, pBR322 DNA of unusually high degrees of negative supercoiling has been isolated from *topA* mutants of E. coli and Salmonella typhimurium; furthermore, the presence of this highly negatively supercoiled population of pBR322 DNA is dependent on the transcription of the *tetA* gene (Pruss, 1985; Pruss and Drlica, 1986). Second, highly positively supercoiled pBR322 DNA has been isolated from E. coli treated with gyrase inhibitors (Lockshon and Morris, 1983). To explain these observations, we have proposed that the transcription process can simultaneously generate two oppositely supercoiled domains which are differentially acted upon by the two topoisomerases (Liu and Wang, 1987).

The essence of the twin-supercoiled-domain model for transcription is illustrated in Figure 1 for the case of a plasmid DNA. Figure 1a depicts a single RNA polymerase molecule transcribing along a circular plasmid DNA. Because of the double-helix structure of the DNA template, as transcription proceeds it is obligatory either that the transcription ensemble R (including the polymerase, its nascent RNA, and RNA-associated proteins) rotates around the DNA, or that the DNA is rotated around its helical axis. The latter motion leads to the positive supercoiling of the DNA ahead of R and the negative supercoiling of the DNA behind R. For a single transcription unit on a circular template, the opposite supercoils can merge and cancel each other by rotational diffusion of the DNA segment in between. In such a case, the moving RNA polymerase generates two oppositely supercoiled gradients. The degree of supercoiling is highest on DNA segments located proximal to the moving RNA polymerase. If two oppositely oriented genes are being transcribed on the same plasmid, as illustrated in Figure 1b, merging of the positively and negatively supercoiled regions requires, in addition to the rotation of the connecting DNA, the rotation of at least one of the transcription ensembles around the DNA. Therefore transcription would generate two oppositely supercoiled domains if the rotation of R is prevented or subject to a large viscous drag.

Because bacterial DNA topoisomerase I and DNA gyrase act differentially on negatively and positively supercoiled domains (Wang, 1971; Gellert et al., 1976), the relative rates of at least four processes would influence the degree of supercoiling of a DNA inside a bacterium: first, transcription, which generates positive and negative supercoils at equal rates; second, diffusional pathways, which allow the cancellation of positive and negative supercoils and which, on a plasmid at least, are dependent on the number of transcription units and their relative directions of transcription; third, gyrase-catalyzed negative supercoiling, which removes positive supercoils and introduces negative supercoils; and fourth, DNA topoisomerase I-catalyzed relaxation of negatively super-

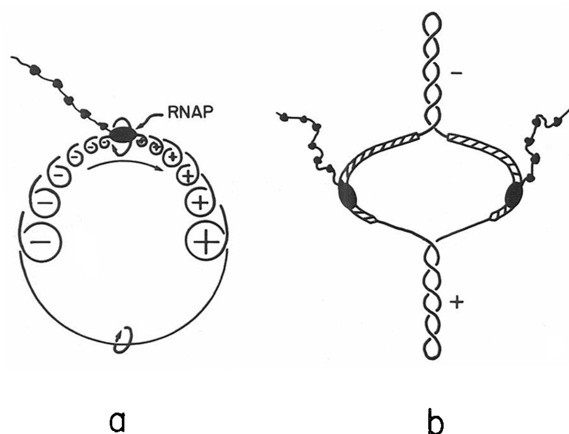

Figure 1. Schematic Illustrations of the Formation of Twin Supercoiling Domains during Transcription

(a) Shown is a single RNA polymerase (RNAP) transcribing along a plasmid. The translocation of the polymerase together with its nascent RNA and RNA-associated ribosomes or ribonucleoproteins generates a positively supercoiled domain (+ signs) in front of the ensemble and a negatively supercoiled domain (− signs) behind it. The positive and negative supercoils can cancel each other by the rotation of the DNA connecting the two domains. (b) When two opposing transcripts are present on the same circular DNA, the positively (+) and negatively (−) supercoiled regions cannot merge by rotating the DNA alone: one of the transcription ensembles must be rotated as well. The supercoiled regions are depicted arbitrarily in the toroidal (a) or interwound (b) configuration.

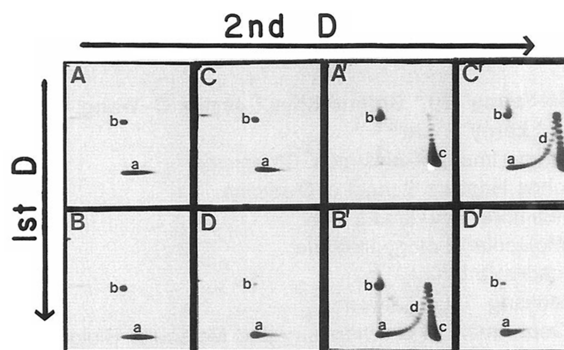

Figure 2. Two-Dimensional Gel Electrophoresis Showing the Formation of Positively Supercoiled pBR322 DNA following Inhibition of DNA Gyrase by Novobiocin, and the Effect of Rifampicin Treatment

E. coli strain AS19 cells harboring pBR322 were grown to log phase and treated with novobiocin or with rifampicin and novobiocin. Plasmid DNAs recovered from the lysates were analyzed by two-dimensional electrophoresis in a 1% agarose gel and were detected by autoradiography of the gel after in situ hybridization with ^{32}P-labeled pBR322 DNA (see Experimental Procedures). (A) Control pBR322 from untreated cells; *a* indicates highly negatively supercoiled DNA, and *b* indicates nicked DNA. (A′) DNA from cells treated with 80 μg/ml novobiocin for 30 min prior to lysis; the bulk of the covalently closed topoisomers ran as a cluster of positive supercoils denoted by *c*. A cluster of positively supercoiled pBR322 dimers, which was partially resolved from the nicked monomer (*b*), was also present. (B′–D′) Prior to the addition of novobiocin, rifampicin was added to 5 μg/ml (B′), 15 μg/ml (C′), or 45 μg/ml (D′). After 30 min novobiocin was added and the cells were subsequently lysed as in (A′). The arc *d* between *a* and *c* contained topoisomers with linking numbers in between those of *a* and *c*. (B–D) Controls of (B′–D′), respectively; samples were recovered from cells treated with rifampicin only.

coiled DNA. While another type I topoisomerase in E. coli, DNA topoisomerase III (Dean et al., 1983; Srivenugopal et al., 1984), might also contribute to relaxation, evidence indicates that this enzyme may be ineffective in vivo (Bliska and Cozzarelli, 1987).

A critical test of the transcription-driven DNA supercoiling model can therefore be carried out by manipulating the processes described above. In this communication we present evidence in support of the model.

Results

Inhibition of Transcription by Rifampicin Abolishes the Positive Supercoiling of pBR322 in E. coli following the Inhibition of DNA Gyrase

In agreement with the observations of Lockshon and Morris (1983), the addition of increasing amounts of novobiocin, an inhibitor of E. coli DNA gyrase, progressively increases the linking number of intracellular pBR322 DNA. As depicted in Figure 2A, pBR322 recovered from untreated cells is resolved by two-dimensional gel electrophoresis into two regions: a spot denoted by *b* consisting of the nicked form of the plasmid, and a streak of spots denoted by *a* consisting of negatively supercoiled topoisomers (for discussions on the interpretation of the two-dimensional electrophoretograms, see Wang et al., 1983; Peck and Wang, 1983; Lee et al., 1981). The same plasmid recovered from cells treated with novobiocin at 80 μg/ml for 30 min gave the pattern shown in Figure 2A′: the negatively supercoiled topoisomers are absent, and a new cluster of positively supercoiled topoisomers denoted by *c* appears. This new cluster of topoisomers has been identified as highly positively supercoiled DNA (data not shown).

The average specific linking difference (superhelical density) of the cluster *c* is estimated to be around +0.03, using two different methods. In the first experiment, samples of pBR322 DNA isolated from untreated cells, from novobiocin-treated cells, and after complete relaxation of the DNA by calf thymus DNA topoisomerase I in vitro were centrifuged to equilibrium in a CsCl density gradient containing 100 μg/ml ethidium bromide. With respect to the position of the relaxed sample, the untreated sample banded at a position 1.5 mm closer to the center of rotation, whereas the sample from novobiocin-treated cells banded at a position 0.8 mm farther from the center of rotation. These data indicate that the plasmid sample from novobiocin-treated cells is positively supercoiled, with an absolute value for specific linking difference of about 0.8/1.5, or about one-half of that of the sample from untreated cells (Radloff et al., 1967). In the second experiment the conditions of two-dimensional gel electrophoresis were modified to resolve the individual topoisomers in cluster *c* (Figure 3). Using pBR322 topoisomers relaxed in vitro by calf thymus DNA topoisomerase I as a reference (denoted by *d* and *e* in Figures 3A and 3B), the average linking number of the cluster *c* is higher by about 10. The

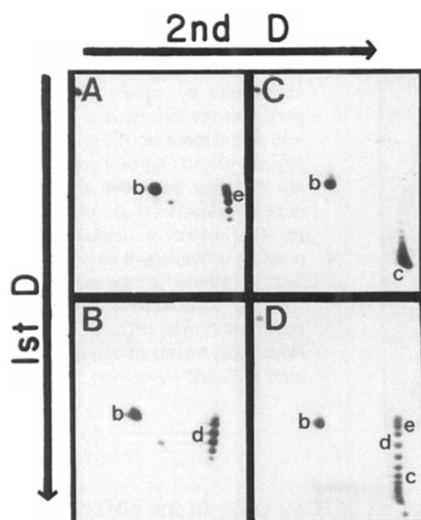

Figure 3. Quantitation of the Degree of Positive Supercoiling of DNA from Novobiocin-Treated Cells

Part of the plasmid DNA recovered from novobiocin-treated cells (see Figure 2A', cluster *c*) was relaxed with excess calf thymus DNA topoisomerase I at 37°C for 1 hr. The pBR322 DNA relaxed in vitro (A and B) and pBR322 DNA recovered from novobiocin-treated AS19 cells (C and D) were loaded separately on 1% agarose gels. The first dimension of electrophoresis was carried out either in ½× TPE at room temperature (A and C) or in TBE containing 5 mM $MgCl_2$ at 4°C (B and D). The second dimension of electrophoresis was carried out in ½× TPE containing 15 μM chloroquine at room temperature.

average linking number of relaxed pBR322 is 4363/10.5 or 416; thus the specific linking difference of the cluster *c* is +10/416 or +0.024.

The formation of the positively supercoiled cluster *c* occurs rapidly. Within 10 min of novobiocin treatment, the species was already present at a high level. Increasing the drug concentration to above 80 μg/ml led to little additional accumulation of this material; treatment with the drug at substantially lower levels gave topoisomers with lower average linking numbers and broader distributions of linking numbers (data not shown). The formation of highly positively supercoiled plasmid DNA during gyrase inactivation appears to be a general phenomenon, which is observed not only in E. coli AS19 cells but also in other E. coli strains such as HB101 and JM101 treated with a higher novobiocin concentration (e.g., 500 μg/ml) (data not shown).

If treatment with novobiocin is preceded by treatment with rifampicin, an inhibitor of RNA polymerase, the amounts of the positively supercoiled topoisomers are reduced. Figures 2B', 2C', and 2D' show the patterns of the topoisomers from cells to which increasing amounts of rifampicin were added 30 min before the addition of novobiocin. At a rifampicin concentration of 5 μg/ml (Figure 2B') or 15 μg/ml (Figure 2C'), the cluster *c* is trailed by a long tail of topoisomers of lower linking numbers. At a rifampicin concentration of 45 μg/ml, which is sufficient to inhibit RNA transcription completely in AS19 cells (Pato et al., 1973), the cluster *c* is no longer formed upon subsequent treatment of the cells with novobiocin (Figure 2D'). Surprisingly, at this high concentration of rifampicin, novobiocin treatment did not even cause much relaxation of the supercoiled DNA (compare Figures 2D and 2D'). The controls for the samples shown in Figures 2B'–2D' are displayed in the corresponding Figures 2B–2D; positively supercoiled topoisomers are not formed by the addition of rifampicin alone. However, at the highest rifampicin concentration the supercoiled state of form I pBR322 DNA (Figure 2D, cluster *a*) was slightly reduced.

Positive Supercoiling Induced by Gyrase Inhibition Is Enhanced by Transcription of the Plasmid in *cis*

To show that the positive supercoiling of a plasmid upon inhibition of gyrase is directly related to the transcription of the molecule itself, we examined the topoisomer distributions of pJW270, a plasmid depicted in Figure 4a. In addition to the replication origin region *ori* of pBR322, this plasmid contains a 1.1 kb segment encoding the *lac* repressor (the *lacI* gene), and the segment of pBR322 encoding the determinant of resistance to tetracycline (the *tetA* gene). The promoter of the *lacI* gene in this plasmid carries an I^Q up-mutation, and the promoter of the *tetA* gene has been replaced by the promoter *lacUV5*, which is repressed by the *lac* repressor.

The linking-number distribution of topoisomers of pJW270 recovered from untreated cells of E. coli strain AS19 is shown in panel A of Figure 4b. The streak of topoisomers denoted by *a* are negatively supercoiled monomeric pJW270 molecules, and the slower-migrating streak of topoisomers to the left of the nicked monomeric pJW270 (denoted by *b*) are negatively supercoiled dimeric pJW270 molecules. Spot *g* corresponds to monomeric pJW270 molecules that had been irreversibly denatured during isolation of the DNA. When cells harboring the plasmid are grown in the presence of isopropyl thiogalactoside (IPTG) to derepress the transcription of *tetA*, highly positively supercoiled monomers and dimers are formed upon inhibition of DNA gyrase (Figure 4b, panel D). The groups of topoisomers show a clear shift toward lower linking numbers if IPTG is omitted in the experiment (Figure 4b, panel C). These results show that transcription of the plasmid, rather than the level of transcription of chromosomal genes, affects the positive supercoiling of the plasmid when gyrase is inhibited. The induction of the *lacUV5* promoter by IPTG has little effect on the linking numbers of the topoisomers if gyrase is not inhibited (compare the patterns shown in panels A and B of Figure 4b).

Further support for the idea that the positive supercoiling of a plasmid induced by inhibition of DNA gyrase is directly related to the transcription of the plasmid in *cis* is obtained by comparing the topoisomer patterns of pBR322 and its deletion derivatives. Figures 5C and 5F depict, respectively, the topoisomer patterns of pBR322 isolated from untreated and novobiocin-treated cells. Figures 5B and 5E depict, respectively, the topoisomer patterns of plasmid pBR322ΔP*tet* from untreated and novobiocin-treated cells. pBR322ΔP*tet* is identical to pBR322 except that several base pairs in the *tetA* pro-

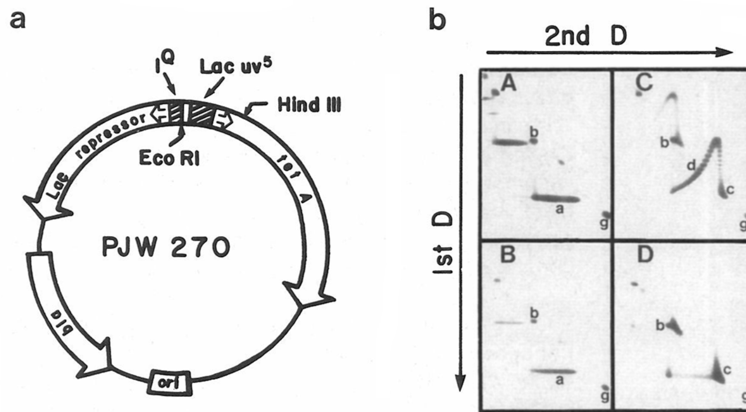

Figure 4. Construction and Supercoiling of pJW270

(a) Schematic diagram of pJW270, which was constructed by replacing the EcoRI–HindIII portion of the *tetA* gene promoter in pBR322 with an inducible *lacUV5* promoter, and inserting an 1100 bp fragment containing the entire *lac* repressor gene with an I^Q promoter up-mutation (Giaever et al., 1988).

(b) The extent of positive supercoiling of pJW270 is dependent on the induction of the *lacUV5* promoter on the plasmid. (A) Control; no IPTG or novobiocin treatment. (B) IPTG treatment (1 mM IPTG for 30 min) only. (C) Novobiocin treatment only. (D) Treatment with both IPTG and novobiocin.

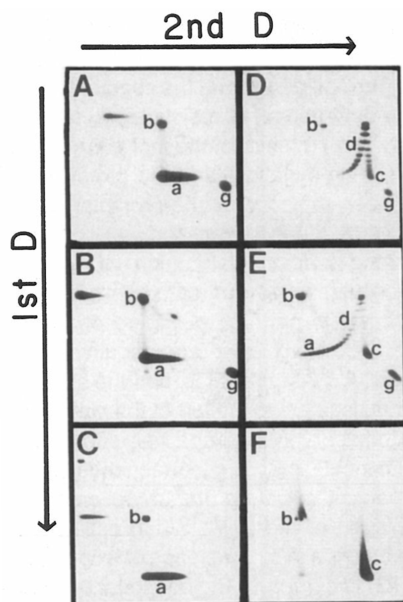

Figure 5. Promoter Mutations in pBR322 DNA Reduce the Highly Positively Supercoiled DNA Population in Novobiocin-Treated E. coli AS19 Cells

E. coli AS19 cells harboring pBR322, pBR322ΔP*tet*, or pBR322Δ-(EcoRI–PstI) were treated with novobiocin (80 μg/ml) for 30 min. (A–C) pBR322Δ(EcoRI–PstI), pBR322ΔP*tet*, and pBR322 DNAs, respectively, isolated from untreated control cells. (D–F) show the corresponding DNAs isolated from novobiocin-treated cells.

moter have been deleted. A comparison of the patterns shown in Figures 5E and 5F shows a clear shift of the topoisomers toward lower linking numbers upon inactivation of the *tetA* promoter. Such a shift is also observed when the PstI–EcoRI fragment of pBR322, which contains the promoter-proximal half of the *bla* (amp^R) gene, is deleted (Figure 5D).

The Extent of Positive Supercoiling Induced by Gyrase Inhibition Is Affected by the Orientation of the Transcription Units on the Plasmid

The population of highly positively supercoiled plasmid DNA is also dependent on the orientation of the transcription units on pBR322 DNA. In the pBR322 genome the *tetA* and *bla* transcription units are oriented in opposite directions. The *rom* transcription unit, which is located between the *ori* and the *tetA* gene, is transcribed in the opposite direction to the *bla* gene (see Figure 6A for the plasmid map). When the *tetA* transcription unit is inverted by inverting the EcoRI–AvaI fragment (pBR322TI1) (Figure 6B), the population of highly positively supercoiled pBR322TI1 DNA in novobiocin-treated cells is reduced. When both the *tetA* transcription unit and the major part of the *rom* transcription unit are inverted by inverting the EcoRI–PvuII fragment (pBR322TI2) (Figure 6C), the population of highly positively supercoiled plasmid DNA is further reduced. These results suggest that a pair of oppositely transcribed genes can greatly affect plasmid DNA supercoiling.

The Formation of Twin Supercoiled Domains by Transcription Is Also Revealed by the High Degree of Negative Supercoiling of Certain Plasmids Isolated from *topA* Mutants of E. coli

It was previously observed that pBR322 molecules isolated from *topA* mutants of E. coli and S. typhimurium were highly negatively supercoiled (Pruss, 1985; Pruss and Drlica, 1986). This high degree of negative supercoiling was shown to depend on the transcription of the *tetA* gene: deletion of the *tetA* promoter or various segments in the *tetA* region was found to suppress the large reduction in the linking number of the plasmid in a Δ*topA* genetic background (Pruss and Drlica, 1986).

We have previously attributed this phenomenon to the generation of twin supercoiled domains by transcription: in the absence of DNA topoisomerase I, only the positively supercoiled domain is efficiently relaxed (by gyrase), and a net accumulation of negative supercoils ensues (Liu and Wang, 1987).

In agreement with this interpretation, we found that the presence of the *tetA* transcription unit per se is not necessary for the formation of highly negatively supercoiled topoisomers in a Δ*topA* strain. The plasmid pDR540, for example, exists in a highly negatively supercoiled form in a Δ*topA* strain even though it lacks the entire *tetA* transcription unit (result not shown). Futhermore, when the

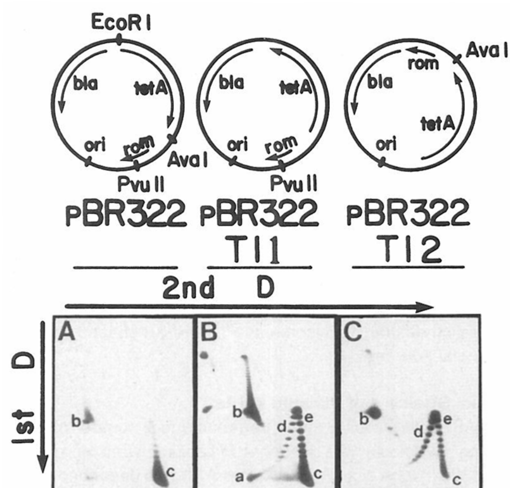

Figure 6. The Extent of Positive Supercoiling of Plasmid DNA following Inhibition of DNA Gyrase Is Dependent on the Orientation of the Transcription Units on the Plasmid

pBR322 (A), pBR322TI1 (B), and pBR322TI2 (C) DNAs were isolated from novobiocin-treated E. coli AS19 cells and analyzed by two-dimensional gel electrophoresis. The orientations of the *tetA*, *bla*, and *rom* transcription units on each plasmid DNA are diagrammatically shown in the plasmid maps above the two-dimensional gel patterns.

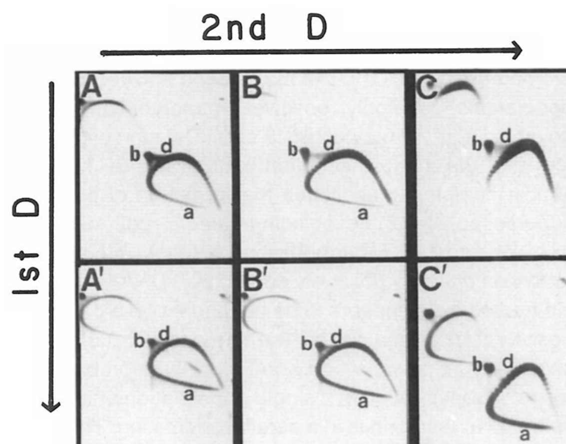

Figure 7. The Formation of Hyper-Negatively Supercoiled Plasmid DNA in E. coli Strain DM800 Δ*topA* Is Dependent on the Orientation of the Transcription Units

Plasmids pBR322TI1, in which the EcoRI–AvaI segment of pBR322 has been inverted (A); pBR322TI2, in which the EcoRI–PvuII segment of pBR322 has been inverted (B); and pBR322ΔP*tet* (C) were each isolated from E. coli DM800 Δ*topA* cells and analyzed by a modified two-dimensional gel electrophoresis procedure. Chloroquine was added to 15 μM in ½× TPE buffer during the first dimension of electrophoresis, and to 60 μM in ½× TPE during the second dimension. For each experiment pBR322 was used as a control (A′–C′).

distribution of topoisomers of pBR322, pBR322TI1, and pBR322TI2 isolated from E. coli DM800 Δ*topA* is compared, it is clear that the inversion of a segment containing the *tetA* transcription unit in pBR322 causes a significant decrease in the degree of negative supercoiling of the plasmid in the Δ*topA* strain (Figure 7).

Discussion

The twin-domain model of transcriptional supercoiling predicts that the local degree of supercoiling of a DNA molecule inside a bacterium is dependent on the level of transcription, the diffusional pathways that allow the cancellation of positive and negative supercoils, and the differential actions of the DNA topoisomerases in their relaxation of the positively and negatively supercoiled domains (see Introduction). The diffusional pathways are in turn dependent on the attachment of the transcription ensemble or the DNA to other macromolecules or macromolecular structures, and, in the case of a circular plasmid with multiple transcription units, on the relative orientations of the units.

Because of the differential actions of the bacterial topoisomerases on positively and negatively supercoiled domains, the generation of such domains by transcription would be manifested by a large change in the linking number of a plasmid DNA when one of the two major topoisomerases, DNA topoisomerase I or gyrase (DNA topoisomerase II), is inactivated. The results presented here demonstrate clearly that changes in the linking number of a plasmid due to the inactivation or absence of a topoisomerase, whether it is the positive supercoiling of a plasmid upon inactivation of gyrase or the hyper-negative supercoiling of a plasmid in a DNA topoisomerase I mutant, are directly related to the level of transcription on the plasmid and to the relative orientations of the transcription units.

In general, these results are consistent with the predictions of the twin-supercoiled-domain model of transcription, and provide strong support for the model. However, a quantitative analysis of the phenomenon of template supercoiling during transcription is difficult because of the kinetic complexity of the problem and the possible effects of DNA replication and protein synthesis. Even for the relatively simple plasmid pBR322, there are at least six promoters. Two of these promoters, the rightward P*tetA* (from which the *tetA* message starts) and a nearby leftward promoter of unknown function, are located on the EcoRI–AvaI restriction fragment near the EcoRI end; the leftward promoter P*bla*, which initiates the *bla* (amp^R) message, is located on the EcoRI–PstI restriction fragment; and three additional promoters, two rightward ones (where the *rom* message and a 104 nucleotide regulatory RNA start), and one leftward one (where the RNA that primes DNA replication originates), are located in the replication origin region of the plasmid (Sutcliffe, 1979; Stuber and Bujard, 1981; Tomizawa and Som, 1984). The relative strengths of the promoters in vivo is unknown, and a population of intracellular plasmid molecules represents a heterogeneous collection in terms of their states of transcription.

The heterogeneous topoisomer distribution of plasmid DNAs isolated from novobiocin-treated cells may reflect plasmid DNA populations with different transcriptional states. For example, the residual highly positively supercoiled pBR322TI1 DNA isolated from novobiocin-treated cells is probably due to the presence of the *rom* transcrip-

tion unit in the opposite orientation to the *bla* and *tetA* transcription units. pBR322 DNA derivatives lacking the *rom* promoter (e.g., pUC19, pAT153) indeed showed reduced populations of highly positively supercoiled DNA in novobiocin-treated E. coli AS19 cells. Deletion of both the *tetA* and the *rom* transcription units from pBR322 DNA almost completely abolishes the formation of positively supercoiled DNA in novobiocin-treated E. coli AS19 cells (H.-Y. W. and L. F. L., unpublished results). Although the formation of highly positively supercoiled DNA in novobiocin-treated cells appears to be dependent on a pair of oppositely transcribed genes, the formation of partially relaxed DNA (clusters *d* and *e* in Figure 6) is probably the result of transcription of a single transcription unit or multiple transcription units in a parallel orientation. The residual moderately negatively supercoiled DNA (cluster *a* in Figure 6) may reflect a transcriptionally inert population of plasmid DNA.

The kinetic complexity due to the heterogeneous presence of multiple transcripts is compounded further by the distribution of topoisomerase binding sites along the DNA. Mapping of the oxolinic acid–induced sites of cleavage by gyrase on intracellular pBR322 indicates that these sites are fairly evenly distributed, but a higher density of stronger sites is present in the *tetA* region as compared with the *bla* region (Lockshon and Morris, 1985; O'Connor and Malamy, 1985). The higher density of gyrase cleavage sites in the *tetA* region may explain the unexpected result that the deletion of the *bla* promoter of pBR322 DNA had almost no effect on the hypersupercoiled population of pBR322 DNA in E. coli DM800 cells (unpublished results).

Our present results suggest that RNA transcription can significantly affect the superhelical state of intracellular DNA. According to our twin-domain model of RNA transcription, the supercoiled state of intracellular DNA is expected to be modulated by a number of parameters such as the level and distribution of topoisomerases, the size and orientation of the transcription units, the relative activity of multiple transcription units, and the presence of large anchorage structures. Local negative supercoiling is expected to be the highest near the promoters. Such localized DNA supercoiling may be sufficiently high to drive DNA structural transitions and affect DNA functions. For long transcription units, the high level of positive supercoiling ahead of the moving RNA polymerase ensemble may also affect further movement of the RNA polymerase. In this case, topoisomerases may function to relieve the superhelical tension and to allow transcription elongation to occur at a high rate.

Whether template supercoiling accompanies transcription in eukaryotes is not readily demonstrable because of the nondiscriminatory nature of the eukaryotic DNA topoisomerases toward positively and negatively supercoiled DNA substrates. There have been a number of studies implicating a role of the topoisomerases in the elongation step of transcription in eukaryotes (Uemura and Yanagida, 1986; Brill et al., 1987; Gilmour and Elgin, 1987; Stewart and Schütz, 1987; Zhang et al., 1988; for earlier studies see the review by Wang, 1985). It seems likely that template supercoiling by RNA transcription occurs in eukaryotes as well. The physiological significance of the effect of transcription-induced template supercoiling on DNA structure and function both in prokaryotes and eukaryotes remains to be established.

Experimental Procedures

Enzymes and Chemicals

Calf thymus topoisomerase I was purified according to Liu and Miller (1981) with minor modifications. Rifampicin, chloroquine diphosphate, IPTG, and novobiocin were purchased from Sigma Chemical Co., and enzymes other than topoisomerase I were purchased from various commercial sources.

Bacterial Strains and Plasmid DNAs

E. coli AS19, an E. coli B strain permeable to a number of antibiotics including novobiocin, was described in Lockshon and Morris (1983). E. coli DM800 (Δ*topA* Δ*cysB gyrB225 acrA13*) was described in DiNardo et al. (1982).

Plasmid pJW270 was constructed by inserting an 1100 bp fragment containing the *lacI* gene, encoding the *lac* repressor (Giaever et al., 1988), into the EcoRI site of pBR322; the *tetA* promoter was also replaced by a *lacUV5* promoter in pJW270. In this plasmid, transcription of *lacI* is from an I^Q promoter in a direction opposite to that of the *lacUV5* transcript. The plasmid pDR540, which contains the *galK* gene under the control of the inducible *tac* promoter, was purchased from Pharmacia. pBR322TI1 was constructed by inverting the pBR322 EcoRI–AvaI fragment, containing the *tetA* transcription unit, after the conversion of the 5′ protruding ends to blunt ends by repair with the Klenow fragment of E. coli DNA polymerase I. pBR322TI2 was similarly constructed by inverting the EcoRI–PvuII fragment of pBR322. pBR322ΔP*tetA*, which contains a small deletion in the *tetA* promoter of pBR322, was constructed by religation of S1-digested pBR322 DNA that had been linearized by digestion with HindIII. pBR322Δ(EcoRI PstI) contains a large deletion between the EcoRI and PstI restriction sites of pBR322; the promoter and part of the transcribed region of the *bla* gene are missing in this derivative.

Cell Growth and Plasmid DNA Isolation

E. coli AS19 cells were transformed with various pBR322 derivatives and grown in Luria broth. Drug treatments were performed when cells were in log-phase growth. Unless otherwise indicated, novobiocin treatment was done with 80 μg/ml novobiocin in the culture at 37°C for 30 min. The following methods have been used for the isolation of plasmid DNAs and shown to produce plasmid DNAs without major differences in their linking numbers: First, the culture was rapidly mixed with a calculated volume of 90°C Luria broth to raise the temperature to 70°C instantaneously. After 10 min of incubation at 70°C, cells were pelleted and plasmid DNA was isolated by lysis with $NaDodSO_4$ (Rowe and Liu, 1984). Second, an equal volume of an alkaline $NaDodSO_4$ solution (0.2 M NaOH, 1% $NaDodSO_4$) at room temperature was added directly to the culture, and plasmid DNAs were isolated by precipitation with KOAc and banding in CsCl–ethidium bromide (Giaever et al., 1988). Third, plasmid DNAs were isolated from pelleted E. coli cells by the alkaline lysis method (Maniatis et al., 1982). Most of the plasmid DNAs reported in this work were isolated by the alkaline lysis method. Since the growth state of cells affects the supercoiled state of plasmid DNA significantly, all experiments were carried out under similar growth conditions.

Two-Dimensional Gel Electrophoresis

Unless otherwise indicated, two-dimensional gel electrophoresis was carried out in 1% agarose gels with ½× TPE buffer (Maniatis et al., 1982). After electrophoresis in the first dimension, the 20 × 20 cm gel was soaked in 15 μM chloroquine (in ½× TPE) for 2 hr in the dark. The soaked gel was turned 90° and electrophoresed in the second dimension in ½× TPE containing 15 μM chloroquine.

In Situ Southern Hybridization

Agarose gels were prepared for in situ hybridization by a modification of the published procedure (Shinnick et al., 1975). Briefly, gels were

stained with 0.5 μg/ml ethidium bromide and exposed to UV light to nick closed circular DNA. Gels were then soaked in 0.5 N NaOH, 0.15 M NaCl for 30 min. Following neutralization of the gels by soaking in 0.5 M Tris (pH 8.0), 0.15 M NaCl, the gels were dried onto Whatman 3MM paper using a gel drier. The dried gels were placed in 6× SSC to float off the paper backing, and prehybridization and hybridization were done according to Maniatis et al. (1982).

Determination of the Specific Linking Difference of pBR322 DNA by CsCl–Ethidium Bromide Equilibrium Sedimentation

Five microgram samples of the following pBR322 DNAs were used: pBR322 DNA isolated from untreated E. coli AS19 cells; pBR322 DNA relaxed in vitro by incubating the DNA with excess calf thymus DNA topoisomerase I (in 40 mM Tris [pH 7.6], 100 mM KCl, 10 mM $MgCl_2$, 0.5 mM EDTA, and 30 μg/ml BSA) at 37°C for 30 min; and positively supercoiled pBR322 DNA isolated from novobiocin-treated E. coli AS19 cells. DNA samples were mixed with stock solutions of CsCl and ethidium bromide to give a solution with a density of 1.56 g/ml and an ethidium bromide concentration of 100 μg/ml. Centrifugation was at 32,000 rpm in a Beckman SW60 Ti rotor for 72 hr (20°C). The relative band positions (the band position of the pBR322 DNA relaxed in vitro was taken as zero) of the various DNAs were determined from the photographs of the gradients under UV illumination; the presence of nicked plasmid DNA in each gradient served as an internal marker.

Acknowledgments

We thank Mr. Hui Zhang and Dr. Mary-Ann Bjornsti for helpful discussions, and Mr. Yeou-Ping Tsao for technical assistance. This work was supported by National Institutes of Health grants GM27731 (to L. F. L.) and GM24544 (to J. C. W.). Part of this work was conducted while three of the authors were visiting at the Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan. The support of the Academia Sinica and the National Science Council (Republic of China) is gratefully acknowledged. J. C. W. was a Guggenheim fellow for the academic year 1986–87, and L. F. L. is a recipient of an American Cancer Society faculty research award.

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Received January 8, 1988; revised March 4, 1988.

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