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Dynamics of DNA supercoiling by transcription in *Escherichia coli*  
(DNA gyrase/topoisomerase I/topology/gene expression)

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ABSTRACT The relative rotation between RNA polymerase and DNA during transcription elongation can lead to supercoiling of the DNA template. However, the variables that influence the efficiency of supercoiling by RNA polymerase *in vivo* are poorly understood, despite the importance of supercoiling for DNA metabolism. We describe a model system to measure the rate of supercoiling by transcription and to estimate the rates of topoisomerase turnover in *Escherichia coli*. Transcription in a strain lacking topoisomerase I can lead to optimal supercoiling, wherein nearly one positive and one negative superturn are produced for each 10.4 base pairs transcribed. This rapid efficient supercoiling is observed during transcription of membrane-associated gene products, encoded by *tet* (the gene for tetracycline resistance) and *phoA* (the gene for *E. coli* alkaline phosphatase), when the genes are oppositely oriented. Replacement of *tet* by *cat*, the gene from Tn9 encoding resistance to chloramphenicol, whose gene product is soluble in the cytosol, reduces the efficiency of supercoiling by RNA polymerase. In a wild-type topoisomerase background, both gyrase and topoisomerase I are kinetically competent to relieve superturns produced by transcription. These results suggest that the level of DNA supercoiling *in vivo* is probably determined by topoisomerase activity, not by transcription.

Transcription can lead to localized supercoiling of DNA because the topology of the elongation complex requires a relative rotation between RNA polymerase and DNA (1, 2). Experimental evidence for this model was originally based on two observations concerning the topology of plasmid pBR322 in *Escherichia coli*: (i) inhibition of DNA gyrase results in the formation of positively supercoiled plasmids (3); and (ii) transcription of *tet* in *Ato* strains leads to the accumulation of a heterogeneous population of pBR322 topoisomers, some of which are hypernegatively supercoiled (4). These seminal observations led Liu and Xiang (2) to propose that the required relative rotation of RNA polymerase about DNA (1) generates positive supercoils downstream from and negative supercoils upstream of supercoiling RNA polymerase. Many studies have verified the essential features of this model both in *vitro* and in bacteria and yeast (5–10). An important conclusion from these studies is that topoisomerases function as swivels to relieve torsional stress during transcription (11).

Since transcription is a ubiquitous process in cells, a fundamental biological question is the extent to which transcription determines the level of DNA supercoiling *in vivo* (5, 12, 13). The mechanistic factors which influence the efficiency and extent of localized supercoiling during transcription, however, are not well understood. First, the forces which may anchor RNA polymerase *in vivo* and thus necessitate the rotation of DNA through the transcription ternary complex have not been clearly identified. Frictional drag on polymerase with its nascent RNA and associated ribonucleo-
oprotein complexes in the viscous environment of the cell may be sufficient to cause supercoiling of the template (1, 2). Alternatively, polymerase may rotate freely about the DNA unless it is directly attached to some larger cellular structure. Second, those superturns which are produced during transcription may migrate rapidly by torsional diffusion many kilobases (kb) away from the site of transcription. Since transcription naturally generates equal numbers of supercoils of opposite sign, torsional diffusion may lead to the rapid equilibration of the superhelix density along a DNA molecule. Finally, topoisomerases may relieve localized superhelical stress so quickly that effects of transcription on DNA topology are normally not apparent. This last possibility implies that the superhelix density of DNA is effectively determined by the activity of topoisomerase enzymes, which has been the traditional view of the control of DNA supercoiling, particularly in eubacteria (14, 15).

The data presented here provide a quantitative basis for evaluating the efficiency of supercoiling by RNA polymerase and the activity of topoisomerases in *E. coli*. In a topoisomerase I-deficient strain of *E. coli*, we show that RNA polymerase can produce superturns at a rate which approaches the maximal efficiency of one positive and one negative superturn per 10.4 base pairs transcribed. This efficient supercoiling is observed during transcription of membrane-associated gene products. Transcription of soluble gene products reduces the efficiency of supercoiling by RNA polymerase. In a wild-type strain of *E. coli*, we demonstrate that both topoisomerase I and gyrase are kinetically competent to relieve supercoils introduced by RNA polymerase. The mechanism of anchoring RNA polymerase during transcription and the consequences of topoisomerase activity for chromosomal supercoiling are discussed.

**MATERIALS AND METHODS**

**Bacterial Strains and Plasmids.** The strains used in this study were RL88, a topoisomerase I-deletion mutant (Δ*tonB*-cysB (Δ206) his-68 gykA223 tyrA2 galK2 malA1 xyl-7 man-2 str’ (rpsL125)) (from B. Bachmann, Yale University), and D1210, a lacIQ derivative of H101 (from N. Linderoth, UC Berkeley). In RL88, lacIQ was provided in trans by pRG1 (16).

To make plasmids which differed only by the orientation of an inserted gene, the double-stranded DNA oligomer

5′-TA TCT AGA GGG CCC CTC GAG-3′  
3′-AGA TCT CCC GGG GAG CTC AT-5′

containing *Xho I* and *Xba I* sites was inserted in both orientations into the Nde I site of pBR322 to yield two molecules, pBRXXopp and pBRXXXpar. Fusions of *phoA* (*E. coli* alkaline phosphatase) bounded by *Xho I* at the 5′ end and *Xba I* at the 3′ end (see below) were then ligated into

Abbreviation: IPTG, isopropyl β-D-thiogalactoside.

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pBRXXopp and pBRXXpar to generate a pair of molecules, designated pXXopp and pXXpar. To make a strongly expressed tet–phoA pair, a tet (tetracycline resistance) gene fusion to the T7A1 promoter and RBSII translation initiation sequence was also constructed (see below) and used to replace the 5' portion of tet in pXXopp and pXXpar. These molecules, designated pNPopp and pNPpar, are shown in Fig. 1. To reverse the orientation of tet in the pNP plasmids, the EcoRI–Sph I fragment of pBRXXopp and pBRXXpar was cut, its ends filled in with the Klenow fragment of DNA polymerase, and religated to yield plasmids in which the constitutive tet was in reverse orientation from that on pBR322. A phoA fusion was inserted at the Xho I and Xba I sites and the 5' portion of tet was then replaced as above to yield pNP0tr and pNP0pr, with strongly expressed phoA and tet in opposed and parallel orientation, respectively. pMCopp and pMCPpar were similarly derived by replacing the entire tet coding sequence of pXXopp and pXXpar to the Mscl site of pBR322 with a cat (chloramphenicol acetyltransferase) gene fusion analogous to the earlier tet fusions.

All gene fusions were constructed in the transcription-translation vector pUHE212 (provided by N. Linderoth; made in the laboratory of H. Bujard, University of Heidelberg) by PCR amplification and modification of phoA, cat, and tet. Care was taken to maintain a direct fusion with the phase T5-derived RBSII ribosome binding sequence, since optimizing translation as well as transcription was an important goal. These fusions resulted in minor changes in the N-terminal sequence of each gene. Each of these fusions proteins retained its biological activity as evidenced by the ability of our Tet and Cat fusion polypeptides to confer antibiotic resistance and the ability of the PhoA fusion product to hydrolyze p-nitrophenylphosphate.

**Topoisomerase Analysis.** Distribution of topoisomerases were trapped by following the method of Okazaki for quenching enzymatic processes in cells (17). Cultures were grown to mid-logarithmic phase (OD600 = 0.5) and isopropyl β-D-thiogalactoside (IPTG) was added to a final concentration of 0.5 mM to derepress transcription. Transcription and other metabolic processes were arrested by the addition of 1 vol of 75% EtOH/2% phenol buffered with 21 mM NaOAc, pH 5.3, and 2 mM EDTA. Cells were spun down from this solution, and DNA was worked up by using standard miniprep protocols (18, pp. 121–126).

Topoisomerase gels (15 × 20 cm) consisting of 1.2% agarose in 0.5X Tris/phosphate/EDTA buffer (ref. 18, p. 8.23) were run at 2 V/cm for 24 hr at 4°C with constant recirculation of buffer. Chloroquine diphosphate was used at the concentration indicated in the figure legends. Two-dimensional gels were run in the first dimension as above, soaked with fresh buffer containing chloroquine at the appropriate final concentration for 5 hr, and run in the second dimension for an additional 10–12 hr.

**RESULTS**

**Model Plasmids for Transcription-Dependent Supercoiling.** Several plasmids were constructed with the goal of optimizing the rate at which RNA polymerase supercoils the template (Fig. 1A). Repressible, strongly expressed genes were juxtaposed in either parallel or opposed orientation. Strong expression was achieved by cloning genes downstream from a modified T7 A1 promoter flanked by operator sequences for the lac repressor (19) (Fig. 1B). Translation of each gene depends on the ribosome binding sequence RBSII from phase T5, which confers a high level of translation (20). Genes were selected whose protein products are targeted to the inner membrane (tet) or periplasmic space (phoA) of *E. coli*. Transcription (4, 5) and translation (21) of tet has been previously shown to be essential for the production of hypernegatively supercoiled species of pBR322 in *topA* mutants. Lodge et al. (21) have postulated that membrane insertion of the nascent tet gene product during transcription is essential for hypernegative supercoiling of pBR322. However, their experiments did not rule out the possibility that translation is required because ribosomes contribute to the hydrodynamic drag on the transcription apparatus.

Strong expression of genes on pNPopp and pNPopp should lead to continuous transcription by RNA polymerase and to formation of topological domains. If polymerase is efficiently anchored, transcription of genes in opposed orientation on pNPopp will trap positive supercoils formed downstream and negative supercoils formed upstream from transcription (Fig. 1A). This buildup of DNA supercoils can be relieved only by topoisomerases. Transcription of genes in parallel orientation on pNPpar introduces positive and negative superturns into each topological domain such that rotation of the DNA helix about its axis can mitigate the buildup of supercoils.

**Kinetics of Supercoiling During Transcription of Membrane-Associated Gene Products.** To measure the kinetics of supercoiling of plasmids pNPopp and pNPpar, the change in linking number, ΔLk, was measured by gel electrophoresis after the induction of transcription in an *E. coli* strain, RL88, that lacks the gene for topoisomerase I. In bacteria, the division of labor between topoisomerases is such that topoisomerase I removes negative superturns formed upstream from transcription and gyrase removes positive superturns formed downstream from transcription (5). The absence of topoisomerase I should lead to the accumulation of negative supercoils after induction of transcription.

There is a lag of approximately 15–30 sec in the production of supercoils on pNPopp (Fig. 2A). Since induction of transcription of genes under control of the lac repressor is almost instantaneous (22), this lag presumably represents the time required to produce an efficient anchor plus the time it takes for gyrase to act upon the overwound domain of the plasmid. By 45 sec, the topoisomerase distribution of pNPopp is very broad, and a fast-moving upward topoisomerase band can be seen. Fifteen seconds later, only this highly under-
wound band is detected. Supercoiling by transcription in this experiment occurs more than 10-fold faster than previously reported (3, 10). This experiment demonstrates that RNA polymerase can, under certain circumstances, form an effective barrier to the diffusion of supercoils.

In contrast, supercoiling of the parallel plasmid is much less efficient (Fig. 2B). At times as long as 10 min there is a heterogeneous distribution of underwater topoisomer. The slow supercoiling of the parallel plasmid is not due to inhibition of transcription, however, since mRNA production from phoA and tet is 2–3 times higher on pNPpar compared with pNPopp as measured by Northern blot analysis (unpublished data), and the copy number of pNPpar and pNPopp is the same (unpublished data). Taken together, these results indicate that torsional diffusion of supercoils on pNPpar must be fast relative to gyrase turnover.

To demonstrate that supercoiling by transcription depends only on the orientation of phoA and tet, plasmids have been constructed in which the orientation of tet is reversed compared with the plasmids shown in Fig. 1A. The same rapid supercoiling is observed only when tet is arranged in opposite orientation to phoA, not when the two genes are parallel (unpublished data). Thus, it is the relative orientation of phoA and tet, not their orientation with respect to some other locus on the plasmid, such as amp, which determines the efficiency of supercoiling by transcription.

The rate of supercoiling of pNPopp has been estimated by using a two-dimensional topoisomerase gel to separate the products at the 45-sec time point (Fig. 3). The least negatively supercoiled bands in this sample have a superhelix density identical to the distribution before addition of IPTG (unpublished data), while the most negatively supercoiled bands have a mobility characteristic of the fast-moving, underwater species produced after 1 min of transcription (Fig. 2A). Between the 45- and 60-sec time points the most positively supercoiled species in the distribution after 45 sec is completely converted to the highly underwater form seen at 60 sec. By counting topoisomerase bands, we can estimate the maximum change in linking number, ΔLkmax, for a plasmid during this interval and thus arrive at a rate for the introduction of superturns by RNA polymerase. Every fifth topoisomer is denoted with a hash mark in Fig. 3 up to ΔLk = -65. The gel loses the ability to resolve the most underwater topoisomer, giving rise to a smear at the tail end of the distribution. We estimate that there are 20 or more topoisomers in this smear. With an estimate of ΔLkmax = -85 in ≤15 sec, a rate of supercoiling by RNA polymerase of ≥5.7 negative superturns per sec can be calculated. Since this rate is the result of transcription of two genes, the rate of supercoiling for one gene can exceed 2.8 superturns per sec. Taking an average elongation rate of 40 nucleotides per sec, RNA polymerase is introducing supercoils at a rate which is greater than 70% of the maximal theoretical rate. Furthermore, assuming that neither topoisomerase III nor topoisomerase IV is involved in relaxing positive supercoils produced by transcription, this rapid change in linking number indicates that DNA gyrase can work with a high efficiency in vivo.

**Superciling During Transcription of a Soluble Gene Product.** Plasmids pNPopp and pNPpar were designed with the idea that concerted transcription, translation, and membrane insertion should be sufficient to effectively anchor RNA polymerase (21). To test whether membrane insertion is necessary, we have constructed similar plasmids in which tet has been replaced by the coding sequence for cat, the gene from Tn9 encoding resistance to chloramphenicol. Since the cat gene product is a cytosolic protein, these constructs test whether two membrane-associated gene products are required for efficient supercoiling by RNA polymerase.
A two-dimensional gel of the topoisomerase distributions of pMCpar and pMCopp 1 min after the induction of transcription is shown in Fig. 4. pMCopp produces an arc of topoisomers ranging from the superhelical density of uninduced plasmid to a highly underwound species reminiscent of the product band seen for pNPopp in Fig. 2A. In contrast, pMCpar is underwound to a much smaller extent. Production of highly underwound pMCopp 1 min after induction of transcription demonstrates that anchoring of polymerase does not depend absolutely on transcription of a membrane-associated gene product. However, supercoiling of pMCopp is far less efficient than for pNPopp, as measured by the yield of underwound DNA produced after 1 min. Interestingly, the topoisomer distribution of pMCopp remains largely unchanged between 1 and 30 min after induction of transcription (unpublished data). This occurs in spite of the fact that expression of Cat protein is linear with time even 30 min after induction, and Cat protein is expressed at a high level, accumulating to 4% of total soluble protein during this interval (unpublished data). The possible significance of this observation is discussed below.

Efficiency of Topoisomerase I and Gyrase in a Wild-Type Strain. To test the efficiency of topoisomerase I and gyrase, the time course of supercoiling was studied in a wild-type topoisomerase strain containing plasmids pNPpar and pNPopp (Fig. 5). All of the topoisomerases migrate as negative supercoils due to the low concentration of chloroquine used in these gels; an increase in mobility after transcription has been induced indicates an increase in negative supercoiling. Two things are apparent upon inspection of these gels: (i) Each plasmid topoisomerase distribution undergoes a change in Lk of about −10 after induction of transcription; this new steady-state Lk is established within 1 min after induction and does not change over the next 10 min. (ii) There is no differential change in topology between opposed and parallel plasmids. We interpret this ΔLk to be a result of polymerase binding and opening a transcription bubble which introduces about 1.7 positive turns per polymerase elsewhere in the DNA (1). These positive turns are rapidly removed by gyrase, and the net result is a decrease in the linking number irrespective of gene orientation. A ΔLk of −10 is equivalent to the binding of about six polymerases per plasmid. This interpretation suggests that both genes on each plasmid are continuously transcribed. In addition, we infer that both gyrase and topoisomerase I efficiently remove transcriptional supercoils, since there is no differential change in linking number between the parallel and opposed plasmids.

DISCUSSION

The observation that transcription can lead to changes in DNA supercoiling has led to speculation that transcription may determine the local superhelicity density in vivo (5) and that genes may be regulated by transcription-induced changes in supercoiling (12, 13). But the in vivo parameters that influence the ability of polymerase to supercoil the template have been largely unknown. These parameters include: (i) the tendency of RNA polymerase to rotate about the template, (ii) the rate at which supercoils are dissipated by torsional diffusion, and (iii) the ability of topoisomerases to remove superturns introduced by transcription. The data presented above provide quantitative insight into these variables.

Kinetics of Supercoiling by RNA Polymerase. We have observed efficient supercoiling by transcription on a plasmid with strongly expressed, membrane-associated gene products arranged in opposed orientation. We define "efficient supercoiling" to mean that the rate of supercoiling for each gene approaches 1 superturn per 10.4 base pairs transcribed and that the plasmid population undergoes a concerted change in supercoiling during a brief (approximately 30 sec) time interval. In a ΔtopA genetic background, superturns accumulate in plasmid pNPopp at a rate of ≥5.7 per sec (Fig. 3); the entire population of plasmids is extensively underwound in the interval between 30 and 60 sec after induction of transcription (Fig. 2A).

Our data also provide insight into the rate of diffusion of supercoils produced by transcription. With a parallel arrangement of genes, torsional diffusion can lead to the annihilation of supercoils, since positive and negative supercoils are produced in a single topological domain. If torsional diffusion were slow in vivo due to interactions of DNA with proteins or other cellular factors, then rapid supercoiling should occur independent of gene orientation. But superturns accumulate at a much slower rate on the parallel plasmids than on the opposed ones (Figs. 2 and 4), and so torsional diffusion must
be fast relative to the rate at which gyrase removes superturns in vivo.

Plasmid pMCopp, containing cat in place of tet, is less efficiently supercoiled than pNPopp by the criteria outlined above. Although some plasmid molecules are extensively underwound 1 min after inducing transcription, the bulk of the plasmid population is underwound only to an intermediate superhelical density on this time scale (Fig. 4). Furthermore, the observed topoisomerase distribution does not change significantly between 1 and 30 min after induction of transcription, as assayed by two-dimensional gel electrophoresis (unpublished data). This slow supercoiling of pMCopp is not due to the inhibition of gene expression at long times, however, since Cat protein accumulates linearly during this time interval and the alkaline phosphatase production is similar to that observed for pNPopp (unpublished data).

The forces that immobilize RNA polymerase during transcription in vivo are still obscure. It is tempting to suggest that membrane-associated gene products can anchor RNA polymerase by concerted transcription, translation, and membrane insertion of nascent polypeptides (21), but the fact that a fraction of the pMCopp plasmid population is rapidly supercoiled implies that membrane attachment by both gene products is not required to immobilize RNA polymerase. However, the inefficiency of supercoiling at times longer than 1 min on pMCopp presents difficulties for any model which postulates that efficient driven rotation of DNA is an intrinsic property of elongating RNA polymerase (23). With such a model, one would expect to observe rapid, complete supercoiling for any two genes expressed in opposed orientation. It is likely that anchoring of RNA polymerase is a complex function of the local cellular environment of a given DNA molecule.

Activity of Topoisomerase I and Gyrase in Vivo. The results presented here provide quantitative evidence for the kinetic efficiency of topoisomerases in coping with supercoiling by transcription. In experiments with strain RL88, ΔKLₘₐₓ is due to DNA gyrase, since topA is deleted. The rapid change in linking number of pNPopp, ≈5.7 superturns per sec (Fig. 3), indicates that gyrase can work with a high turnover rate in vivo. This result is somewhat surprising in light of the gyrA223 compensatory mutation in RL88. Compensatory mutations normally decrease the intrinsic activity of gyrase (24, 25). This rate is significantly faster than in vitro estimates of about 0.5—1 turnover per sec (equivalent to 1—2 superturns per sec) (26). It is possible that there is more than one gyrase acting on each plasmid in vivo, and our measured rate is therefore not a turnover number per enzyme. It is also conceivable that in a region where positive superturns are rapidly introduced by transcription or replication, slippage by gyrase is reduced, increasing enzyme efficiency (27).

Experiments in a wild-type topoisomerase strain support the notion that both gyrase and topoisomerase I can remove superturns at a rate equal to their introduction by RNA polymerase. In the wild-type strain, if either enzyme lagged behind the rate of supercoiling by RNA polymerase, then the steady-state Lk would differ for the parallel and opposed plasmids. Torsional diffusion can contribute to the relaxation of superturns on the parallel but not the opposed plasmid. Since a differential change in Lk is not observed, we conclude that both topoisomerase I and gyrase effectively relieve the rapid introduction of supercoils by transcription. This, in turn, implies that there is excess topoisomerase activity in E. coli such that a rapid burst of transcriptional activity after induction by IPTG can be accommodated. This result has implications for chromosomal supercoiling, since presumably the topoisomerases respond with equal rapidity to induction of transcription on the chromosome.

The kinetic efficiency of the topoisomerases supports the idea from many other studies that these enzymes make up a homeostatic system for the maintenance of superhelical density around an optimal set point in E. coli (24, 25, 28, 29). It further suggests that it is the topoisomerases—and not DNA-tracking processes such as transcription—that are the primary determinants of the level of DNA supercoiling in E. coli. Supercoiling by polymerase could be transiently evident during the time between induction of a gene and the arrival of topoisomerase I or gyrase at a supercoiling hot spot. Thus, fluctuations in superhelical density, as opposed to stable changes due to transcription, may be important in a variety of biological situations. Consistent with this idea, B- to Z-DNA transitions have been reported upstream of the tet gene on pBR322 in the presence of wild-type topoisomerases (7). Presumably these structural transitions occur on a subset of plasmid molecules for which gyrase, but not topoisomerase I, has removed superturns generated by transcription. If supercoiling by transcription is a transient phenomenon, then one might expect its effects to be most relevant to biological processes that occur infrequently. Lodge and Berg (30), for instance, have presented evidence that transposition of Tn5 is favored at sites upstream of the tet gene. Since transposition is necessarily a rare event, it might be preferentially stimulated by localized fluctuations in supercoiling. General recombination may also be sensitive to fluctuations in supercoiling due to transcription.

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