Transcription regulates oxolinic acid-induced DNA gyrase cleavage at specific sites on the *E.coli* chromosome

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ABSTRACT

Prominent DNA gyrase-mediated cleavage sites, induced by oxolinic acid, occur at specific, but infrequent, locations on the *Escherichia coli* chromosome. These sites, which we call toposites, may represent high affinity DNA gyrase binding sites or may mark chromosomal regions that accumulate superhelical stress. Toposites are usually grouped in 5 to 10 kb clusters that are mostly 50 to 100 kb apart. The total number of clusters on the chromosome is between 50 and 100. The location of sites depends on the local sequence. The extent of DNA gyrase cleavage at toposites can be strongly modulated by transcription occurring at as far as 35 kb away.

INTRODUCTION

The chromosome of *E. coli* is segregated into separate supercoiled domains (1). The number of domains was estimated *in vitro* (1) and *in vivo* (2) to be between 40 and 100 by the number of nicks required to relax the chromosomal DNA completely. A domain structure is also apparent in electron microscope pictures of the *E. coli* nucleoid (3). However, the identification of factors that define and maintain supercoiled domains has been elusive as has the identification of specific domain boundaries.

The existence of separate supercoiled domains potentially allows the superhelical density to vary on different parts of the same chromosome. In *E. coli*, DNA superhelicity is maintained by the action of two enzymes with opposite activities: DNA gyrase, a type II topoisomerase (4) and DNA Topoisomerase I (5). Topoisomerase I relaxes DNA, while DNA gyrase introduces negative supercoils. In eukaryotic cells it has been suggested that Topoisomerase II binding sites anchor chromosomal domains to the nuclear matrix (6).

DNA gyrase, an A_2B_2 tetramer, acts by passing a DNA helix through a transient double-stranded DNA break (7). The treatment of a DNA gyrase–DNA complex with either oxolinic or nalidixic acid prevents the religation reaction and leads to the formation of a relaxation type complex (8). The location of DNA gyrase is revealed when such a complex is treated with a protein denaturant, because separation of the enzyme subunits, in the presence of inhibitor, results in a permanent double stranded DNA break. Although cleavage induced by oxolinic acid occurs at DNA gyrase binding sites (9), not all binding sites are cleaved when oxolinic acid is added (10). It is not clear whether addition of inhibitor prompts DNA gyrase binding at specific sites.

At least seventy inhibitor-promoted DNA gyrase cleavage sites, of variable magnitude, were identified on plasmid pBR322 (11, 12). Although analysis of the pBR322 cleavage sites uncovered only a dinucleotide consensus sequence (12) the occurrence of preferred sites suggests that the enzyme must recognize a specific sequence. Results obtained in sedimentation velocity experiments suggested that DNA gyrase cleavage sites occur on the *E. coli* chromosome at about 100 kb (13) or 12 to 25 kb (14) intervals. The latter study also concluded that DNA gyrase cleavage sites are randomly distributed around the replication terminus region as has a recent study of a 10 kb region around the *gyrB* gene (15). Thus, prior to the study presented here, it was believed that, in bacteria, topoisomerases acted at random locations to maintain normal levels of chromosomal superhelicity.

DNA supercoiling is influenced, *in vivo*, directly and indirectly, by such environmental factors as temperature (16), osmolarity (17), anaerobiosis (18), and growth transitions (19). Supercoiling itself specifically affects a variety of cellular processes including DNA replication, transposition, recombination and transcription (20). The initiation of transcription of many genes is sensitive to supercoiling (21–24), while the transcription process itself appears to unwind DNA, producing local and temporal changes in superhelicity (25, 26). Thus, one might expect that DNA gyrase cleavage sites should be associated with transcription or other biological functions influenced by DNA supercoiling. However, until this study no biological functions were associated with any DNA gyrase cleavage sites in *E. coli*.

Pulsed field gel electrophoresis (PFG) and large DNA technologies allow the purification, manipulation and analysis of intact chromosomal DNAs (27, 28) and were used to construct a low resolution *Not* I, *Sfi* I and *Avr* II restriction map of *E. coli* (29, 30). These methods, and results, permit molecular events to be monitored, in context, directly on the chromosome (31,32).

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Here, they were used to analyze the distribution of DNA gyrase cleavage sites on the intact *E. coli* chromosomal DNA *in vivo* and the factors that influence this cleavage. These experiments identify specific, but infrequent, DNA gyrase cleavage sites that appear to sense transcription.

METHODS

Strains, plasmids and media

E. coli strains used were EMG2 (wild type(F^+, λ^+); 33), W3110 (*thy*A36, *deoC*2, λ^-, F^-, INV [*rrnD-rrnE*]: 34), AA787 (Δ[*relA-argA*]216, *thr*-1, *leuB6*, *pth*-1, *his*-65, *argH*46, *thi*-1, *gal*-3, *malA*1, *xyl*-7, *mtl*-2, *tonA2*, *supE*44: 35) and MO1518 (*thr*-1, *ara*-14, *leuB*6, Δ[*gpt-proA*]62, *lacY*1, *tsx*-33, *supE*44, *galK2*, *hisG4*, *rfbD1*, *mgl*-51, *rpsL*31, *kdgK*51, *xyl*-5, *mtl*-1, *argE3*, *thi*-1, *recB*21: 36). The plasmids used as probes in hybridization experiments were pMT521 (37) containing the *lps* gene and located at 0 min, pK12 (38) containing the *phoA* gene located at 9 min, pLC18-42 (39) containing the *rec*A gene located 58 min, pTK201 (40) containing the *gly*S gene located 79 min, pPP1 (41) containing the *pyrE* gene located at 82 min, and f12 (42) containing part of the *repFIC* sequences of F plasmid. Plasmid pCD1 contains a 1.6 kb *Pst* I-*Not* I fragment subcloned into plasmid pUC8 from phage λ3E3 (43). Plasmid pCD1 is a half linking clone located at the proximal end of *Not* I fragment **C** at 7 min (this study). Plasmid pK12 is located on *Not* I fragments **C** (this study). See (27) for the assignment of other cloned sequences to *Not* I restriction fragments.

Preparation of chromosomal DNA

Cultures grown in M9 medium or L broth (44) to a density of about 2×10^8 cells/ml were treated with 50 μg/ml oxolinic acid, usually for 10 min, before harvesting. For induction experiments, 0.2% fucose, 0.2% sorbitol, 0.2% lactose or 1 mM IPTG was added to cells grown in M9 medium. The *phoA* operon was induced by first growing cells in A medium (45) with phosphate and shifting them with one wash step to the same medium without phosphate.

Chromosomal DNA was prepared and digested in agarose inserts as described (28, 27). Briefly, chilled cells were centrifuged at 4000 rpm for 5 min, washed in 1/3 volume of Pett IV (10 mM Tris-HCl (pH 7.6) 1M NaCl), centrifuged at 4000 rpm for 5 min, resuspended in 1/8 volume of Pett IV, mixed with an equal volume of 1% low gelling agarose and poured in 100 μl molds. The resulting agarose inserts were then incubated for 24 hr in a lysing solution (0.6 mM Tris-HCl (pH 7.6) 100 mM NaCl, 100 mM EDTA (pH 7.5), 0.05% Brij 58, 0.02% deoxycholate, 0.05% Sarkosyl, 1 mg/ml lysozyme, 20 μg/ml RNase), and 1% sodium dodecyl sulfate when samples were treated with oxolinic acid.

DNA separation, blotting and hybridization

DNA was separated using a Pulsaphor apparatus (Pharmacia-LKB). The gels were run in modified TBE (0.1 M Tris base, 0.1M boric acid, 0.2 mM EDTA, pH 8.2) for 40 hr at 10 V/cm unless otherwise indicated. The pulse time was changed according to the separation desired. PFG-separated DNA was transferred to a nylon membrane after 2 min UV nicking as described (27, 28). Probes labeled by random oligonucleotide priming (46) were hybridized to the membrane-bound DNA as described (27, 28).

RESULTS

DNA gyrase cleavage occurs at specific chromosomal locations

Chromosomal DNA from *E. coli* K12 wild type strain EMG2 treated with oxolinic acid and detergent, was purified in agarose, and analyzed by PFG electrophoresis (27, 28). Under these conditions, only a few breaks are introduced into each chromosomal DNA molecule since large (>500 kb) chromosomal fragments were detected (data not shown). Cleavage was dependent on oxolinic acid interaction with DNA gyrase since it was not detected in an oxolinic acid resistant *gyrA* mutant strain nor in a $gyrA_{ts}$ strain incubated at the restrictive temperature (data not shown). The average size of the oxolinic acid-induced fragments seen is much larger than reported previously (13, 14). In the earlier studies, chromosomal DNA samples were prepared in liquid and may have had random shear damage common in solution preparations of very large molecules. All DNA samples used here were prepared in agarose to protect the DNA from shear damage.

The randomness of DNA gyrase cleavage on chromosomal DNA purified in agarose was evaluated by indirect end labeling experiments (47). *E. coli* chromosomal DNA cleaved, *in vivo*, with DNA gyrase, and *in vitro* with the restriction enzyme *Not* I, was fractionated by PFG electrophoresis and hybridized to probes near or at the ends of chromosomal *Not* I fragments (Figure 1). The restriction enzyme *Not* I cleaves the *E. coli* EMG2 genome into 22 fragments ranging in size between 20 and 1000 kb which are readily fractionated by PFG (Figure 1A). Oxolinic acid-induced DNA gyrase cleavage results in a light background smear in the *Not* I digested samples. If DNA gyrase cleavage on the chromosome is random, no specific fragments, other than the uncut parental *Not* I fragments, should be detected in indirect end labeling hybridization experiments. However, multiple specific fragments smaller than the parental *Not* I fragments are detected (Figure 1B). Multiple fragments are detected because DNA gyrase cleavage at each specific site is incomplete. The difference in sizes between the fragments gives the distances between the cleavage sites beginning at the *Not* I site and extending in one direction along the chromosome.

Plasmid pLC18-42 was used as indirect end labeling probe in the experiment shown in Figure 1B. This clone contains the *recA* gene located near the proximal end of the largest genomic *E. coli Not* I fragment (1 Mb: fragment **A**) that spans one fifth of the chromosome from 58 to 81 min (29). Specific DNA gyrase cleavage sites are seen over 450 kb of this fragment in the experiment shown in Figure 1B. An extremely strong site was detected at, what appeared to be (see below) about 180 kb from the proximal end of the *Not* I fragment **A**. Strong cleavage sites often appear in groups of two or three, spaced by 5 to 10 kb intervals. Although each cluster is separated from its neighbors by what appears to be 50 to 100 kb intervals, the spacing and intensity of the DNA gyrase cutting varies from site to site. Longer X-ray film exposures reveal additional bands of much lower intensity. This suggests that DNA gyrase acts at other locations on the chromosome but to a much lower extent.

We call the strong DNA gyrase cleavage sites toposites. Twelve groups of toposites are seen when the entire *Not* I restriction fragment **A** is scanned (data not shown). The distribution of toposites over about 70% of the genome that has been scanned, thus far, by indirect end labeling experiments, is fairly constant (data not shown). Although the amount of cleavage is extremely sensitive to lysis conditions, the distribution is not. Densitometric quantitation of five independent hybridization experiments

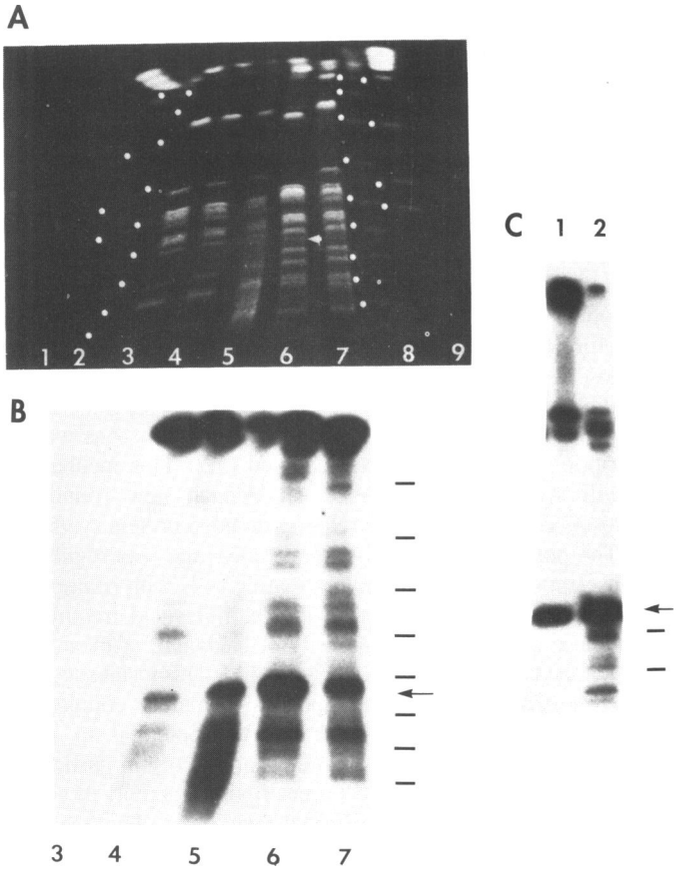

Figure 1. Specific, but infrequent, oxolinic acid induced DNA gyrase cleavage sites on the *E. coli* chromosome and F^+ plasmid. *E. coli* cells were treated with 50 μg/ml oxolinic acid for 10 min, lysed, and genomic DNA was purified and digested with the restriction enzyme *Not* I, and fractionated by PFG using (A and B) 30 s or (C)12 s pulse times as described in Materials and Methods. (A) Ethidium bromide stained gel. Lanes 1 and 9 contain *S. cerevisiae* chromosomal DNAs (positions indicated with white dots). Lanes 2 and 8 contain concatemers of phage lcI_{857} (monomer = 48.5 kb: positions indicated with white dots). Lanes 3, 4 and 5 contain DNA from *E. coli* wild type strain EMG2. In lane 3 the treatment with oxolinic acid was omitted. In lane 5, the cells were treated with lysozyme before embedding in agarose. Lane 6 contains DNA from *E. coli* strain MO1518. This strain contains a $recB^-C^-$mutation (36). Degradation of chromosomal DNA containing double stranded breaks was expected to be lower in a strain lacking the major *E. coli* nuclease. Lane 7 contains DNA from strain W3110. This strain has an inversion between 72 min and 90 min. (B) Hybridization of the gel shown in Figure 1A with plasmid pLC18-42 containing the chromosomal *recA* gene. Bars indicate positions of l ladder size standards. (C) Hybridization of PFG fractionated DNA purified from untreated cells (lane 1) and oxolinic acid-treated cells (lane 2) to an F^+ plasmid sequence present in plasmid f12. The arrow indicates in (A) and (B) the position of the fragment used for the mapping experiments (see Results) and in (C) the position of the once broken F^+ plasmid.

indicated that, on the average, about one third of the *Not* I A fragments are cleaved by DNA gyrase. Seventy five percent of these cleavages occur at very strong sites such as that located 180 kb from the proximal end. On the average, strong sites are cleaved about 16 fold more than weak sites while the ratio of strong to weak cleavages ranges from >40 to >6.

Bejar and Bouche purified oxolinic acid-induced DNA gyrase-cleaved chromosomal DNA via sedimentation through sucrose (14). Hybridization with several chromosomal probes revealed homologous sequences in all fractions. This was interpreted to mean that cleavage was random. However, the same results would be obtained if DNA gyrase cleavage was partial or the subsequent chromosomal samples were subjected to shear damage (see above). Thus, there is clearly no conflict between the results obtained by Bejar and Bouche (14) and the results shown here.

It was of particular interest to examine the chromosome for the occurrence of toposites in regions where the action of DNA gyrase may be especially critical. DNA gyrase is necessary for the initiation of DNA replication (48) and is required for the decatenation of the daughter molecules after DNA replication (49). Thus, one might expect that the origin and terminus of replication might contain a high density of strong toposites or unusually strong ones. However, during logarithmic growth, the density of DNA gyrase sites in these two regions seems to be identical with that of the rest of the chromosome (data not shown).

The very low yield of chromosomal cleavage by DNA gyrase observed in the experiments reported here, coupled with PFG separation, allows a simultaneous quantitative analysis of numerous DNA gyrase cutting sites spanning a long distance along the chromosome. The application of these methods to map DNA gyrase cleavage sites on the chromosome is potentially quite powerful.

Toposites are determined by local sequence

The position of toposites on the chromosome could be dependent on a specific DNA sequence near or at the toposite itself (cis acting), or on other factors such as its distance from the origin of replication. Several lines of evidence indicate that the toposites are determined by the local sequence. *E. coli* strain AA787 has a deletion of the *relA−argA* region located at 60 min. The DNA gyrase cleavage sites in the regions surrounding the deletion were examined in an indirect end labeling hybridization experiment (Figure 2A). Although the strains are not isogenic, the pattern of DNA gyrase cutting sites, measured by intensity and distribution, in the regions surrounding the deletion was, in general, the same in *E. coli* strains AA787 and EMG2 (Figure 2A: compare lanes 2 and 4). This shows that toposites are not defined by their distance from a specific point of the chromosome.

E. coli strain W3110 contains a large inversion between 72 min and 90 min. This inversion involves a fifth of the chromosome. Plasmid pTK201, containing the gene *glyS* located at the distal end of *Not* I fragment **A**, was used as a probe in indirect end labeling experiments to scan the inversion region. In *E. coli* wild type strain, EMG2 this probe detected chromosomal DNA that spanned from 82 min to 74 min, while in strain W3110 DNA was detected that spanned from 82 to 90 min. The pattern of cleavage is identical in these two strains, despite the inversion (Figure 2A: compare lanes 2 and 7). This means that the position of toposites is not affected by the inversion. Again, this argues that toposites are determined primarily by local DNA sequence. This constant and sequence-specific position of toposites suggests that toposites are important functionally.

Modulation of DNA gyrase activity at toposites

Although oxolinic acid-induced DNA gyrase cleavage requires active enzyme (8, 50, 51), past studies have failed to correlate such DNA cleavage with any known biological activity. Thus, it was important to determine whether any cellular functions were associated with DNA gyrase cleavage at toposites. Therefore, cleavage was examined under different growth conditions known to influence the physiology of *E. coli* and thought to lead to perturbations in chromosomal superhelicity.

Higgins *et al.* (17) postulated that a general mechanism for environmental regulation of gene expression, such as osmolarity, might be through supercoiling. Hence, if DNA gyrase activity at toposites maintains chromosomal supercoiling, and high osmolarity affects chromosomal supercoiling, one might expect to detect variations in either intensity or distribution of DNA gyrase cleavage at toposites at different osmolarities. Although no global change in toposite cleavage was detected, cleavage at a few specific toposites appeared to decrease at high osmolarity (Figure 3A). Additionally, few, if any, changes were detected around the *proU* and the *bgl* operons (data not shown), whose regulation is thought to be influenced by osmolarity (17). Thus, these experiments do not reveal any general relationship between DNA gyrase cleavage at toposites and changes in osmolarity. This may mean that the plasmid reporter molecules, used by previous workers, are not good indicators of chromosomal superhelicity, perhaps because their superhelical density is strongly regulated by transcription (26). On the other hand, the effect of osmolarity on the chromosome may be very temporally limited or localized. Of course it is also possible that changes in supercoiling at high osmolarity may not be related to increased DNA gyrase activity at toposites. For instance, they may be mediated in a more complex way, possibly through effects on topoisomerase I.

Although DNA gyrase activity at toposites does not appear to be influenced by the osmolarity of the growth media, it is profoundly affected by the type of growth medium. For instance, large differences both in distribution and intensity of DNA gyrase activity at toposites were observed between *E. coli* grown in rich medium (LB) and minimal medium (M9) with glycerol as a carbon source (Figure 3B). Intensities and distributions of toposites also varied with growth stage (data not shown). Since complex cellular changes occur during such experiments, it is difficult to identify factors that specifically alter the partitioning of DNA gyrase on the chromosome. However, variations in DNA gyrase cleavage, observed previously (14) and in this work, in different growth media or in different growth phases, might be expected to be due to alterations in processes, such as transcription and/or DNA replication, which affect supercoiling. For example, all the amino acid biosynthetic genes are induced when cells are grown in minimal medium, while in rich medium macromolecular operons are expressed at their maximal levels.

Amino acid Starvation and Rifampicin inhibit DNA gyrase cleavage at toposites

Very little DNA gyrase cleavage was detected in a multi-auxotrophic strain that had been starved for amino acids for 90 min before the addition of oxolinic acid, whereas cleavage at toposites resumed within 15 min after removing the block to translation (data not shown). This treatment turns off translation completely, and turns off transcription of those operons where transcription and translation are coupled (52). This incubation also will stop DNA replication since each new round of chromosomal DNA replication requires de novo protein synthesis (31). The possibility that DNA gyrase cleavage was regulated by transcription was examining by treating cells with rifampicin (to inhibit transcription) prior to the oxolinic acid treatment. Under these conditions, the intensity of cleavage, at most, but not all, of the toposites decreases (Figure 4A). This result suggests that DNA gyrase cleavage at toposites is mainly coupled to transcription. Since transcription has the potential to alter supercoiling (26), DNA gyrase binding or activity in particular chromosomal regions may reflect transcriptional activity of genes in the region in response to different conditions. This may be tested directly by examining the behavior of DNA gyrase at cleavage at toposites near particular genes (see below).

Physical mapping and cloning of two toposites

The existence of both the high (43) and low (29, 30) resolution chromosomal restriction maps allows the precise mapping of toposites on the chromosome. Toposites have no known selectable phenotype. However, they can be localized in a library. The first

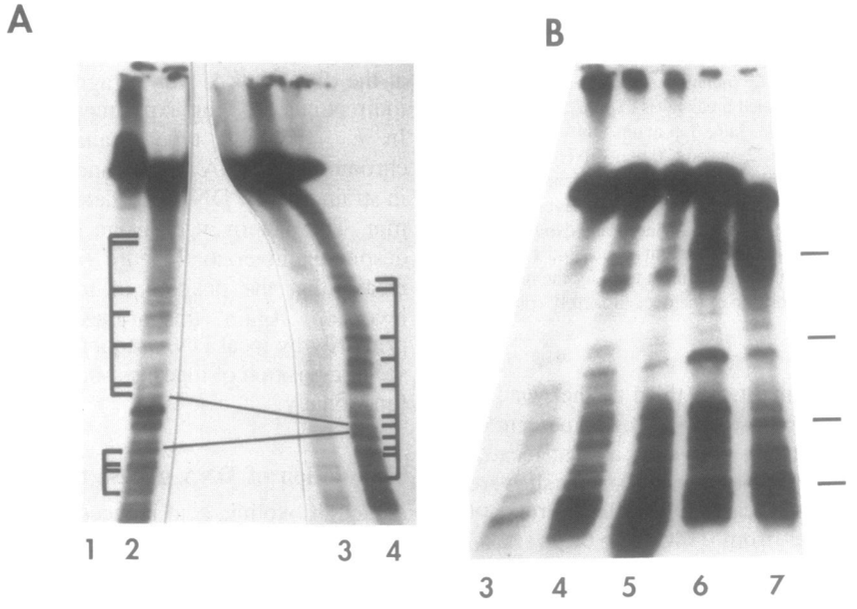

Figure 2. DNA gyrase cleavage at toposites is determined by the local context. (A) Hybridization experiment, using plasmid pLC18-42 as a probe, similar to that described in the legend of Figure 1. Lanes 1 and 2: strain EMG2 not treated and treated with oxolinic acid; lanes 3 and 4: strain AA787 not treated and treated with oxolinic acid. The toposites surrounding the deletion are indicated. (B) Hybridization experiment using plasmid pTK201 as a probe to the PFG gel shown in Figure 1A. Lane numbers correspond to those described in Figure 1. Bars are placed at 100 kb increments.

toposite cloned was the most frequently cut site detected thus far. It is located around 61 min on the genetic map and on *Not* I fragment **A**. The combined DNA gyrase–*Not* I restriction enzyme cleavage experiments create a fragment (*Not* I toposite fragment) that is distinctly separated from all of the parent *Not* I fragments and can even be seen on ethidium bromide stained gels (indicated by an arrow in Figure 1A). This fragment was eluted from a gel, labeled by the random oligonucleotide priming method and hybridized to members of the ordered, overlapping λ library of Kohara *et al.* (43) covering the region around 61 min. A contiguous set of 13 clones (contig) hybridized to the *Not* I-toposite fragment. One end of this contig contains the *Not* I site (phage 8G10), while the other end contains the toposite. Phage 9A12, containing the toposite, was located 140 kb from the *Not* I site whereas the *Not* I-toposite fragment migrated like an 180 kb fragment in PFG. This size discrepancy has several possible origins: (a) DNA mobility might be retarded in PFG because DNA gyrase or a peptide might have remained covalently bound to the DNA despite the proteinase K treatment, (b) DNA in this region might be migrating anomalously in PFG because of the presence of some unusual sequence, (c) there may be a difference between the physical map of *E. coli* strain W3110, the source of DNA for the library (43), and EMG2, the strain used in this study, or (d) the *Not* I physical map, representing average sizes along the chromosome may not be accurately aligned in this region. In fact, a deviation from the average size relationship has been detected between the genetic and the physical map immediately proximal to this region (30). Furthermore, the 62 min region of *E. coli* strain W3110 was recently shown to contain a previously unrecognized rearrangement (53) as well as five IS5 insertions (54). The insertion element IS5 contains bent DNA known to cause anomalous electrophoretic migration (55).

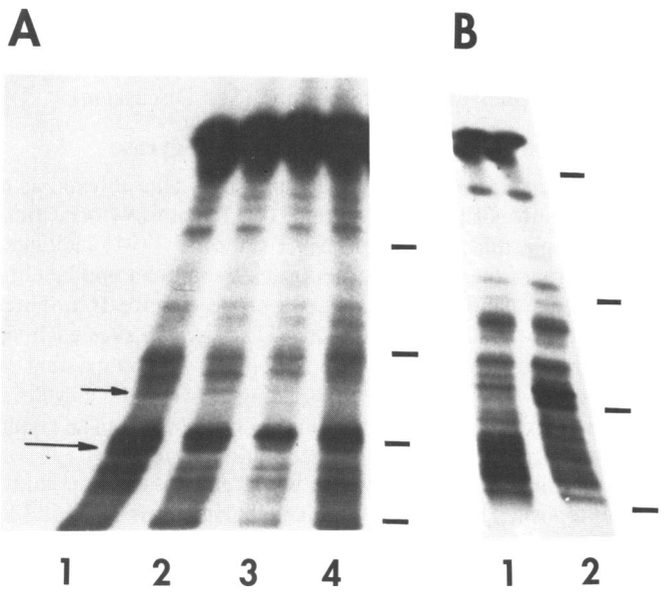

Figure 3. The effect of growth conditions on DNA gyrase cleavage at toposites. Hybridization experiment, using plasmid pLC18-42 as a probe, similar to that described in the legend to Figure 1A except that genomic DNA from *E. coli* strain EMG2 was purified from cells grown and treated with 50 mg/ml oxolinic acid in (A) LB medium, containing no salt (lane 1), 40 mM NaCl (lane 2), 100 mM (lane 3), or 200 mM NaCl (lane 4) or (B) in minimal, M9 medium with glycerol (lane 1) or in rich, LB medium (lane 2). The arrow indicates the few sites that changes in (A). The PFG fractionation used 25 s pulse times. Bars are placed at 100 kb increments.

Restriction fragments from lambda 9A12 were subcloned into pBR322 and ordered according to data available (43). These subclones were used as probes to analyze PFG-separated chromosomal DNA after *Not* I and DNA gyrase cleavage. The most distal subclone that hybridizes to the *Not* I-DNA gyrase

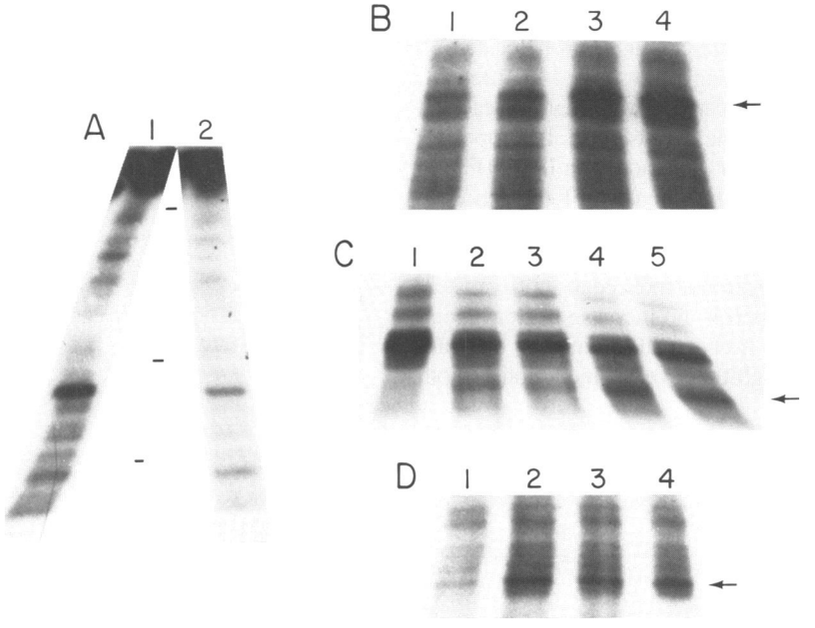

Figure 4. Transcription modulates DNA gyrase cleavage at toposites. Hybridization experiments similar to that described in the legend to Figure 1A except that genomic DNA was purified from *E. coli* strain 2660 (A) treated with oxolinic acid as usual (lane 1) or treated with 15 μg/ml of rifampicin for 30 min before oxolinic acid addition (lane 2) or induced for (B) fucose, (C) lactose or (D) sorbitol. In (B–D) samples were taken before and at 10 min intervals after induction (lanes left to right). Hybridization probes were plasmid pLC18-42 (A,B and D) or plasmid pK12 (C). Arrows indicate toposites that appear to modulated induction of the specific operons.

fragment will contain the DNA gyrase site that created the fragment. This approach allowed us to localize the strongest site on a 2 kb *Bgl* II-*Bam*H I fragment. A second toposite was identified, also on lambda 9A12. It is located about 10 kb from the first toposite. Alignment of these subclones with the restriction map of Kohara *et al.* (43) and other published maps (56) show that one of these two toposites is within the *fucOAPIK* operon and another toposite is located 5 kb downstream of this operon (Figure 5). This alignment allowed us to test directly the effect of transcription of a specific operon on DNA gyrase activity at a specific toposite on the chromosome.

Transcription modulates DNA gyrase cleavage at toposites

DNA gyrase cleavage at toposites on the chromosome was examined in the neighborhood of several operons. The effect of transcription on toposites was first tested with the fucose operon because this operon was located between the two mapped toposites. This operon was induced in *E. coli* cells grown in M9 glycerol medium by the addition of 0.2% fucose. Samples taken at 20 min intervals were treated with oxolinic acid, SDS was added and the DNA purified and digested *in vitro* with the restriction enzyme *Not* I. Hybridization experiments showed that the intensity of the strong toposite located about 5 kb downstream of the *fucPIK* genes greatly increased while the intensity of other sites basically remained unchanged (Figure 4B). Similar major increases, were observed in DNA gyrase cleavage at nearby toposites upon expression of several operons. For instance, major increases in DNA gyrase cleavage downstream of the lactose operon and nearby the sorbitol operon appear after induction of these operons (Figure 4C and 4D, respectively). The position of the sensitive toposite relative to the direction of transcription of the operon appears to be important (see below). Other minor changes were also observed. These are probably due to changes in growth phase in the course of the experiment (see above). The new large DNA technologies allow monitoring of long range interactions between toposites and nearby genes to be monitored directly on the chromosome (see below). For instance, two divergently transcribed operons, *lacZYA* and *phoA*, located 35 kb apart, are both inducible and strongly transcribed (Figure 6A). A strong cluster of toposites is located downstream of each of these operons. An increase in the intensity of the toposites downstream of *lac* can be seen after induction with IPTG (Figure 6B; compare dlanes 1 and 2). The intensity of the toposites downstream of *phoA* also increases after induction by removal of phosphate from the medium but to a lesser extent (lane 3 Figure 6B). However, when the *phoA* operon is induced at approximately the same time as the *lacZYA* operon, no increase in cleavage at the toposites downstream of *lac* is observed (Figure 6B; compare lanes lanes 2 and 4) even though the level of induction of both operons, detected by assaying β-galactosidase and alkaline phosphatase activity, remained unchanged (data not shown).

These experiments show that the activity of DNA gyrase can be influenced over long distances on the chromosome and suggest that transcriptional processes also may interact over long distances. These results show that single clusters of toposites are modulated by specific operon transcription and support some, but not all, aspects of the double domain model for transcription (25) as it applies to the chromosome (See Discussion).

Specific cleavage of plasmid F$^+$ by DNA gyrase

E. coli strain EMG2 contains a F$^+$ plasmid. This allowed us to ask whether toposites occur on this 100 kb, naturally occurring, low copy plasmid. The single *Not* I site on F$^+$ (31) facilitated the analysis of the position of toposites by indirect end labeling experiments using plasmid f12 as a probe (Figure 1C). Three rather evenly spaced groups of two DNA gyrase sites each are seen. This suggests an organization similar to that existing on the chromosome. However, the spacing between the clusters is smaller (30 kb) than the spacing on the chromosome. The results show that toposites can be isolated and analyzed on large low copy plasmids like F or R, which will greatly assist their study.

Can toposites be detected on a small, high copy plasmid like pBR322? The positions of DNA gyrase cleavage sites were

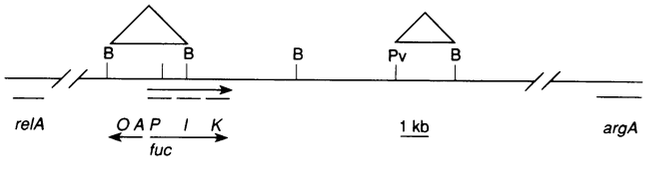

Figure 5. Mapping of two toposites nearby and within the fucose operon. The triangles represent the positions of two toposites on λ phage 9A12 (thick line). The position and the direction of transcription of thr *fucOAPIK* operon as determined by Chen *et al.* (1987) are indicated.

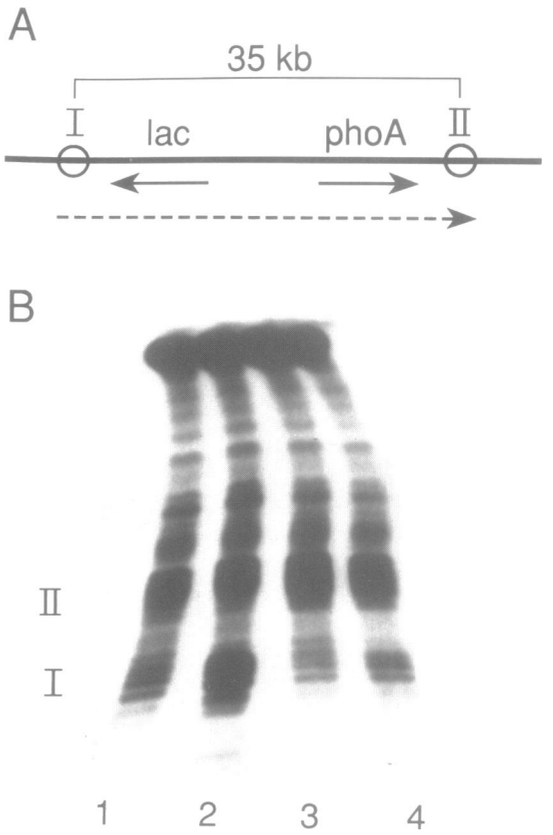

Figure 6. Transcription modulates DNA gyrase cleavage at toposites over long, but limited distances, on the *E. coli* chromosome. (A) The *lacZYA* and *phoA* operon are located 35 kb apart on the chromosome. The location of clusters of toposites is indicated by circles and direction of transcription is indicated by a thin arrow. The dashed line indicates the direction of the DNA replication fork. (B) Hybridization experiment, using plasmid pCD1 as a probe, similar to that described in the legend to Figure 1, except that samples were prepared from uninduced control cells (lane 1) or 30 min after cells were induced for *lacZ* (lane 2), two hours after derepressed for *phoA* (lane 3), or simultaneously derepressed for *lacZ* and induced for *phoA* (lane 4) and fractionated with PFG using 25 s pulse times. Specific growth conditions are described in Materials and Methods.

determined *in vivo*, on two pBR322 derivatives, each containing one of the two toposites flanking the *fuc* operon (see above), as described (11). Surprisingly, although some additional DNA gyrase sites seemed to appear on these plasmids when compared with the parental pBR322, no very strong new ones were detected. This result shows that the DNA sequence of a toposite alone is not sufficient to create a strong toposite on a small multicopy plasmid (see Discussion).

DISCUSSION

Toposites are high affinity DNA gyrase binding sites

Our results reveal the existence and distribution of clusters of high affinity DNA gyrase cleavage sites on the *E. coli* chromosome. Previous work had shown that DNA gyrase-dependent oxolinic acid-induced cleavage of the *E. coli* chromosome generates large DNA fragments. However, specific cleavage sites were not identified. Here we show that cleavage of the chromosome occurs at specific, but infrequent sites, which we have called toposites. The distribution of the toposites on the chromosome appears to be fairly uniform even over the origin and the terminus region of DNA replication during steady growth conditions.

Presumably, these experiments only detect active DNA gyrase, because the addition of oxolinic acid traps a reaction intermediate (51). One would expect that DNA gyrase activity is only required *in vivo* at specific times, at specific toposites, when the local superhelical density needs to be adjusted. Cellular processes which change supercoiling vary spatially and temporally along the chromosome. Thus, the intensity of cleavage may simply reflect a local requirement of DNA gyrase for maintaining the normal chromosomal supercoiling level (see below) and may explain why some DNA gyrase binding sites are not cleavage sites (10).

It is estimated that there are 500−1000 gyrase molecules per cell (M. Gellert, personal communication). The studies presented here estimate that there are around 100 clusters of toposites sites per chromosome. Considering the fact that each cell contains, on average 2.5 chromosomes, it could be that most DNA gyrase molecules are located at toposites. However, it is also possible that DNA gyrase is more widely distributed over the chromosome than at toposites. Franco and Drlica (15) found 24 DNA gyrase cleavage sites on a 10 kb chromosomal DNA fragment covering the *gyrB* region, whereas a survey of the *Not* I fragment **L** (203 kb) that covers the same region revealed the presence of only 6 strong toposites (data not shown). Thus, there appear to be several categories of DNA gyrase sites. The high affinity sites (toposites) described here occur at low frequency. The second category is exemplified by the 70 low affinity sites occurring in pBR322 (11), or by the 24 described by Franco and Drlica (15). The experiments presented here show that the former sites are responsive to transcription, while no functional role has been detected in the latter case. Thus, our experiments underscore the importance of scanning large chromosomal regions, since without such an overview, the existence of transcriptionally sensitive sites would easily be missed.

Specific DNA sequences are necessary for DNA gyrase cleavage at toposites. REP (or PU) sequences are short sequences repeated hundreds of times on the *E. coli* chromosome. They can bind proteins associated with the nucleoid (57), and it has been suggested that REP sequences and their associated proteins could bind DNA gyrase and serve to define the boundaries of a supercoil domain (58, 9). It is unlikely that toposites are specified simply by REP sequences because REP sequences occur much more frequently than toposites. However, toposites could represent a subset of REP sequences defined by a more specific consensus REP sequence, or a cluster of REP sequences since REP sequences are known sometimes to be clustered. The resolution of our mapping method (3 kb) does not allow us to determine whether toposites are a cluster of weak DNA gyrase cleavage sites. Sequencing the cloned toposites should reveal the relationship, if any, of toposites to the REP sequences.

Sequence alone is not sufficient to produce a toposite, since a single toposite cloned onto pBR322 is not expressed. However, this may not be surprising. For instance, cleavage at a specific toposite may require specific coupling to transcription. Even if the primary sequence required for DNA gyrase binding is present, other constraints, possibly supercoiling stress, induced or localized by DNA binding proteins, by a membrane attachment site or by a second toposite, might be required to create high affinity DNA gyrase sites. These unknown important factors apparently exist on large plasmids such as F^+, which also contain toposites. Experiments are underway to reconstruct a functional toposite on a smaller plasmid to facilitate studies of factors that regulate it.

Transcription regulates DNA gyrase activity at toposites

Processes such as transcription modulate DNA supercoiling. DNA gyrase cleavage at toposites shows an overall decrease when transcription of DNA is stopped by addition of rifampicin. This could reflect a general decreases in the DNA gyrase activity to relieve positive supercoils expected to accumulate during transcription. Wu *et al.* (25) recently showed that transcription is accompanied by the accumulation of positive supercoils in front of the transcription complex and negative supercoils behind the complex. This occurs because the RNA polymerase transcription complex cannot rotate freely around DNA or because the DNA is anchored. Thus, during transcription DNA gyrase activity at toposites ahead of an operon might increase in order to relax the positive supercoils generated. A simultaneous decrease in DNA gyrase activity at toposites would be expected to occur behind a transcribing operon. In general, we detect no or only a slight decrease in DNA gyrase cleavage at toposites behind transcribing operons.

Why is there no general decrease in DNA gyrase cleavage at toposites associated with transcription behind operons? The experiments reported here measure DNA gyrase cleavage and do not measure levels of supercoiling. *In vivo*, Topoisomerase I relaxes negative supercoils. Hence, during transcription an increase in the activity of Topoisomerase I, rather than a decrease in DNA gyrase activity, might occur behind the transcriptional apparatus. Furthermore, the chromosomal regions scanned in these experiments contain multiple genes interacting in a complex and unknown manner. For instance, clearly something different happens when both the *lac* and *phoA* operons are induced simultaneously. Although the available data do not reveal a mechanism, the results clearly indicate that the two transcription units are communicating over 35 kb. One possibility is that they are competing for some common anchor point, perhaps some cell surface receptor required for protein transport (see below). A second possibility is presumably some kind of topological coupling between the *lac* and *phoA* operons and/or genes located between the *lac* and *phoA* operons, but it is not clear what kind of coupling could explain the observed results.

Transcription alone is not sufficient to regulate DNA gyrase cleavage at toposites. What other factors are required? Toposites

may represent a DNA–membrane attachment site such as those postulated by Lodge *et al.* (59) that involve a coupling between transcription, translation, and insertion of nascent proteins into the membrane. A membrane coupling might restrict the diffusion of supercoiling changes thereby recruiting DNA gyrase to the vicinity. In such cases toposites need not be determined by a specific DNA gyrase recognition sequence, and membrane coupling does not necessarily have to be in operons that modulate DNA gyrase cleavage at toposites.

Lambda phage DNA infected into *E. coli* is circularized and then supercoiled in the absence of transcription and replication (8). Similarly, DNA gyrase cleavage at toposites is not completely eliminated by inhibiting transcription nor by inhibiting replication (see Figure 4). Thus, it is not likely that transcription alone regulates chromosomal supercoiling or DNA gyrase cleavage at toposites.

E. coli chromosomal organization

It is tempting to speculate that toposites may serve simultaneously as boundaries to superhelical stress and as nucleoid domain boundaries. It is very likely that transcription of several different operons might interact to modulate DNA gyrase activity at the same toposite. If this is the case, correlation of DNA gyrase cleavage at specific toposites with the induction of various genes may be a way to map the boundaries of superhelical domains.

Our results show that there are strong, specific DNA gyrase binding sites at constant positions in each cell. These toposites may define topological domains. This makes it likely that the separate supercoiled domains controlled by DNA gyrase are also situated at specific locations on the chromosome. Although the spacing between strong clusters of toposites (between 50 and 100 kb) is similar to the size expected for supercoiled domains (2), there is no evidence that they are identical. In eukaryotic cells, topoisomerase II (the equivalent of bacterial DNA gyrase) is the major component of the protein scaffold that fastens DNA loops (60). Indeed, if toposites regulate bacterial chromosome structure, the long range organization of prokaryotic and eukaryotic DNA would then be remarkably similar.

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