JOURNAL OF BACTERIOLOGY, Oct. 1988, p. 4983–4985
0021-9193/88/104983-03$02.00/0

Vol. 170, No. 10

Rifampin and *rpoB* Mutations Can Alter DNA Supercoiling in *Escherichia coli*

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Received 17 March 1988/Accepted 19 July 1988

Two cases are described which indicate that RNA polymerase could alter DNA supercoiling. One occurred in a *topA* mutant in which abnormally high levels of plasmid supercoiling were lowered by rifampin, an inhibitor of the β subunit of RNA polymerase. The second case involves suppression of a temperature-sensitive *gyrB* mutation by a rifampin-resistant allele of *rpoB*, the gene encoding the β subunit of RNA polymerase. Measurements of chromosomal DNA supercoiling show that the *rpoB* mutation reduced DNA relaxation.

DNA supercoiling in bacteria is controlled by the activities of two enzymes, DNA gyrase and DNA topoisomerase I (for reviews, see references 4 and 25). Gyrase introduces negative supercoils, and topoisomerase I modulates the effects of gyrase by removing them. The level of supercoiling itself appears to be homeostatically regulated by levels of expression of the genes encoding these two enzymes (13, 14, 23; Y. Tse-Dinh and R. Beran, J. Mol. Biol., in press). Even small changes in supercoiling expected to arise from changes in temperature or intercalating dyes appear to be corrected by topoisomerase action (6, 9). Abnormal levels of supercoiling can, however, be generated by mutations in the genes encoding topoisomerases (16, 19, 20, 21, 24) and by inhibitors of gyrase (5, 11, 12, 18). We report two examples in which DNA supercoiling was influenced by RNA polymerase. The first involves the ability of rifampin to lower the very high levels of plasmid supercoiling seen in a *topA* (topoisomerase I) mutant (16), and the second concerns the suppression of a temperature-sensitive *gyrB* (gyrase) mutation by an *rpoB* (RNA polymerase) mutation (8).

The high levels of negative supercoiling seen in pBR322 when the plasmid is isolated from *topA* mutants (16) depend on the integrity of the *tet* gene: insertion or deletion mutations in the *tet* promoter or in the 5′ coding region of *tet* lower supercoiling (17). To further test the idea that RNA polymerase and transcription are responsible for this phenomenon, we examined the effects of rifampin, an inhibitor of RNA polymerase, on DNA supercoiling. Strain DM800 (3, 19, 22), transformed with pBR322, was grown to mid-log phase in M9 medium (15) and labeled by growth in tritiated thymidine (10 μCi/ml) for about 0.5 cell generations. The cells were then chilled quickly and harvested by centrifugation. For plasmid studies, cells were lysed by incubation with egg white lysozyme followed by dilution into 1.25 volumes of 0.1% Triton X-100–0.05 M EDTA, pH 8. Cellular debris and chromosomal DNA were removed by centrifugation, and the resulting supernatant was deproteinized by incubation with 0.2% sodium dodecyl sulfate–94 μg of proteinase K per ml at 37°C for 30 min. Sedimentation analyses were performed as described previously (5) with various concentrations of ethidium bromide. Each sucrose gradient contained a sedimentation marker (^{14}C-labeled bacteriophage T4B for chromosomal measurements and ^{32}P-labeled bacteriophage lambda restriction fragments for the plasmid measurements). Centrifugation was carried out in a Beckman SW50.1 rotor at 4°C for 30 min at 17,000 rpm for bacterial nucleoids and at 33,000 rpm for 15.5 h for pBR322. Samples were collected from the bottoms of the centrifugation tubes, and the means of the distribution of radioactivity were used to calculate sedimentation rates relative to the internal markers.

Low concentrations of ethidium relaxed negatively supercoiled DNA and lowered the sedimentation rate. The sedimentation rate reached a minimum value at the critical dye concentration, which under the conditions used, is proportional to superhelix density (1). Ethidium concentrations above the critical concentration introduced positive supercoils and increased the DNA sedimentation rate. Plasmid DNA from untreated cells showed a shallow titration curve, with a minimum at about 2.7 μg of ethidium bromide per ml (Fig. 1), while plasmid DNA from rifampin-treated cells exhibited a sedimentation minimum at 1.5 μg/ml and the titration curve was similar to that observed with nucleoids isolated from untreated cells (Fig. 1). Thus, inhibition of RNA polymerase eliminated the very high levels of plasmid supercoiling observed in this *topA* mutant; a similar effect was not observed in plasmids from cells wild type for *topA* (data not shown). The shallow titration curve seen with pBR322 from untreated cells probably reflects the extensive topoisomer heterogeneity observed previously by electrophoretic analysis (16).

The effect of transcription on DNA topology has been explained in the following way (10). Transcription is thought to create topological domains in which positive superhelical tension develops downstream from transcribing genes and negative tension develops upstream. In pBR322, the *tet* and *bla* genes are oppositely oriented, so transcription would tend to divide the plasmid into a positive and a negative domain of supercoiling. The biochemical properties of the topoisomerases suggest that gyrase might normally relieve the positive supercoils and topoisomerase I might relieve the negative ones. Thus, transcription would cause negative supercoils to accumulate in a *topA* mutant, and positive supercoils would accumulate when gyrase is inhibited. An example of the latter case was first reported by Lockshon and Morris (11) and has recently been more fully documented (26). Thus, it is likely that one of the functions of

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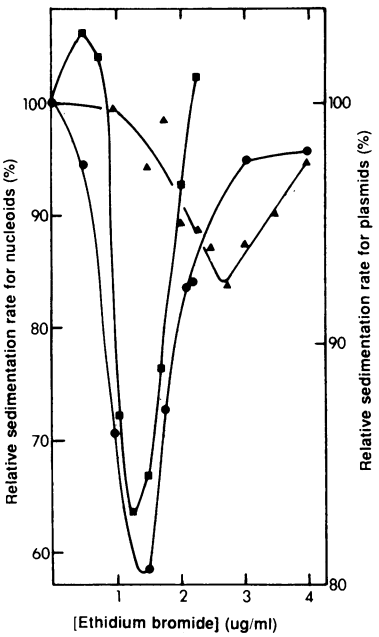

FIG. 1. Effect of rifampin on pBR322 supercoiling. Sedimentation analyses were performed as described previously (5) at the indicated concentrations of ethidium bromide. The means of the distribution of radioactivity were used to calculate sedimentation rates, which are plotted relative to those determined in the absence of ethidium bromide. Symbols: ●, nucleoids from untreated cells; ▲, pBR322 from untreated cells; ■, pBR322 from cells treated with 200 μg of rifampin per ml for 5 min prior to harvesting of cells.

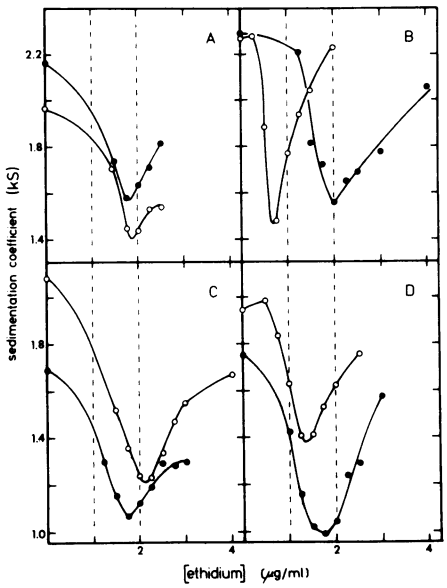

FIG. 2. DNA supercoiling in nucleoids extracted from cells containing gyrase and RNA polymerase mutations. Cultures of *Escherichia coli* cells were grown at 30°C (●) to mid-log phase, ^{3}H-labeled thymidine was added (10 μCi/ml), and half of each culture was shifted to 43°C (○) for 40 min. Cells were rapidly chilled, and they were concentrated by centrifugation. Cells were lysed as described previously (21). Sedimentation analyses were carried out as described in the text by using ^{14}C-labeled bacteriophage T4B (1.025 kS [2]) as a sedimentation marker. (A) Strain EC6 (wild type). (B) Strain EC1510 (*gyrB203 gyrB221*). (C) Strain TS126 (*rpoB410*). (D) Strain EC1524 (*gyrB203 gyrB221 rpoB410*).

topoisomerases is to provide the topological swivels needed for the elongation step of transcription.

The second phenomenon we investigated is an extension of the observation that the spontaneous rifampin-resistant allele *rpoB410* suppresses the temperature-sensitive growth phenotype of the *gyrB41* mutation (*gyrB41* was subsequently resolved into two mutations; *gyrB203*, conferring temperature sensitivity; and *gyrB221*, responsible for resistance to coumermycin [13]). Since one of the characteristics of temperature-sensitive *gyrB* mutations is extensive DNA relaxation following a shift to restrictive temperature (21, 24), we investigated the possibility that suppression by *rpoB410* correlates with reduced DNA relaxation. Chromosomal supercoiling was compared in strains carrying several combinations of the *rpoB* and *gyrB* mutations (Fig. 2). Supercoiling in the wild-type strain EC6 (8) was unaffected by a shift from 30 to 43°C (Fig. 2A). In the temperature-sensitive *gyrB* mutant EC1510 (8), the increase in temperature caused extensive DNA relaxation and thus a reduction in the ethidium concentration required to titrate the chromosomal DNA supercoils (Fig. 2B). A strain containing only the *rpoB410* mutation (TS126, constructed by transduction of rifampin resistance from strain EC1524 [8] into EC6) exhibited a slight increase in supercoiling following the increase in temperature (Fig. 2C). In strain EC1524, in which *rpoB410* mutation has suppressed the temperature-sensitive *gyrB* phenotype (8), the level of chromosomal supercoiling was slightly lowered by the shift to 43°C (Fig. 2D), but the extent of relaxation was much less than that observed in strain EC1510 (Fig. 2B). Thus, the *rpoB410* mutation reduced the amount of relaxation arising from the mutant *gyrB*, and this change in supercoiling probably contributes to the ability of *rpoB41* to suppress the temperature-sensitive phenotype. The mutant gyrase is thought to retain some activity at the restrictive temperature (8), and it may be that the *rpoB* mutation causes an abnormally high level of gyrase gene expression. This speculation has not been tested.

In addition to the two examples described above in which RNA polymerase action raises supercoiling or diminishes relaxation, a case has also been reported in which a *rho* mutation causes relaxation of plasmid DNA supercoiling (7). The molecular basis for this phenomenon has not been established. It could arise from effects of the *rho* mutation on plasmid transcription, from a reduction of gyrase expression (the *rho* mutant contains abnormally low levels of the gyrase subunits [T. Phillips and F. Neidhardt, cited in reference 7]), or from a combination of the two.

Two general ideas emerge from the types of experiment discussed above. First, not all DNA molecules from the same cell need have the same level of supercoiling, because in *topA* mutants transcription in some plasmids leads to much higher levels of supercoiling than is observed in the chromosome (Fig. 1) (17). This would lead us to expect that there might be local differences in supercoiling from one region of the chromosome to another, particularly in *topA* mutants. Second, proteins such as RNA polymerase and Rho may influence supercoiling in a general way through their effects on the expression of gyrase, providing the cell with additional ways to compensate for mutations in the topoisomerase genes.

We thank Marcin Filutowicz for supplying bacterial strains, Gail Pruss for many stimulating discussions, and the following individuals for critical comments on the manuscript: Richard Burger, Marila Gennaro, and John Kornblum.

This work was supported by grants from the National Institutes of Health and the American Cancer Society (NP 565). R.F. and T.S.

were supported in part by a National Institutes of Health Predoctoral Training Grant.

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