DNA Supercoiling and Suppression of the leu-500 Promoter Mutation

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Received 11 June 1985/Accepted 1 August 1985


DNA supercoiling has been strongly implicated as a factor affecting gene expression in bacteria (for reviews, see references 4, 5, 24, and 30). Part of the basis for this phenomenon is probably the fact that negative supercoils facilitate the unwinding of DNA by RNA polymerase and thereby facilitate the initiation of transcription (29). However, changes in supercoiling elicit highly varied responses in gene expression, depending on the specific gene (and promoter). Whereas expression from some promoters is enhanced by negative supercoiling or reduced by DNA gyrase inhibitors, expression from others is unaffected by these manipulations (1, 2, 10, 15, 21, 22, 25, 31). Moreover, expression in some cases, including the genes encoding DNA gyrase, is enhanced by inactivation of gyrase or by reduced levels of supercoiling (3, 12, 18, 21, 27).

Suppression of the leu-500 promoter mutation in Salmonella typhimurium by top mutations (formerly supX [20]) (14) is an example of enhanced gene expression caused by the absence of topoisomerase I activity (for a review, see reference 24). Topoisomerase I relaxes negatively supercoiled DNA in vitro (28) and appears to perform this function in vivo (13, 17–19). Consequently one would expect that increased levels of DNA supercoiling are responsible for suppression of the leu-500 promoter mutation by top mutations. However, the discovery that many Escherichia coli topoisomerase I mutants have acquired secondary mutations (3, 18) which reduce chromosomal DNA supercoiling to levels below that of wild-type strains (18) casts doubt on this explanation. We were interested, therefore, in determining whether increased supercoiling was indeed responsible for suppression of the leu-500 promoter mutation in top mutants.

Pruss (17) initially sought to address this question by comparing the superhelical densities of plasmid pBR322 DNA isolated from top mutants and from their top+ parental strains. This study showed that pBR322 DNA from top mutants is more negatively supercoiled than DNA from top+ parental strains and that restoration of topoisomerase I activity in top mutants returns pBR322 DNA supercoiling to approximately normal. Restoration of topoisomerase I activity in top mutants also reimplies the leucine auxotrophy caused by the leu-500 promoter mutation (11). Although these observations are all consistent with the hypothesis that increased negative supercoiling is responsible for the Leu+ phenotype of leu-500 top mutants, this study additionally revealed that pBR322 DNA supercoiling is not always an indicator of bacterial chromosome supercoiling (17). Therefore, we sought a different way to test the hypothesis that increased DNA supercoiling suppresses the leu-500 mutation in top mutants. We reasoned that we could test this hypothesis by seeing whether some manipulation (other than restoration of topoisomerase I activity) which reduced DNA supercoiling would also eliminate top suppression of the leu-500 promoter mutation. We chose the antibiotic coumermycin A₄ for this purpose (lot 77F437; obtained as a gift from Bristol Laboratories, Syracuse, N.Y.). Since coumermycin prevents the introduction of superhelical turns into DNA by DNA gyrase (7), it seemed possible to find a concentration of the drug which would reduce intracellular levels of supercoiling activity, without being lethal, and would create the desired reduction in DNA superhelicity. Coumermycin, an inhibitor of the gyrB subunit of DNA gyrase, seemed preferable to inhibitors of the gyrA subunit for such an experiment, since the covalent gyrase-DNA complexes created by inhibitors of the gyrA subunit (6, 26) might create additional problems for cell growth. Therefore, we investigated the effect of low doses of coumermycin on growth of a leu-500 top mutant in the presence and absence of leucine.

We tested drug concentrations in the range of 0.25 to 5 μg of coumermycin per ml. A typical experimental protocol, that of the experiment shown in Fig. 1, was as follows. A culture of the leu-500 Δtop mutant PM233 (11, 14) containing pBR322 (17) was grown overnight at 37°C without aeration in M9 medium containing 1 mM MgSO₄, 0.04 mM CaCl₂, 0.4% glucose, 80 μg of L-cysteine per ml, 40 μg of L-tryptophan per ml, 35 μg of ampicillin per ml, and 20 μg of tetracycline per ml. This overnight culture was diluted 100-fold into four portions of the same medium containing 40 μg of leucine per ml, 2.5 μg of coumermycin per ml, both, or neither. To achieve an identical concentration in the two portions of medium containing coumermycin, cells were first diluted into medium which contained 2.5 μg of coumermycin per ml and no leucine. These diluted cells were then divided equally into separate flasks, and leucine was added to one flask. The coumermycin used was from a solution freshly made in dimethyl sulfoxide; an equivalent concentration of dimethyl

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sulfoxide was present in the medium lacking coumermycin. The diluted cells were grown with aeration at 37°C, and growth was monitored by measuring the increase in turbidity with a Klett-Summerson colorimeter. After approximately 27 h, half of the culture with coumermycin but without leucine was transferred to a separate flask, and leucine was added. Aeration of both cultures was continued at 37°C.

Cells inoculated into minimal medium without leucine but with 2.5 μg of coumermycin per ml failed to grow significantly even when incubated for 76 h at 37°C (Fig. 1A). The apparent very slow rate of growth in this culture was actually not caused by an increase in cell density but by a yellowing of the growth medium due to tetracycline (data not shown). This culture still showed no growth by 94.5 h at 37°C (data not shown). In contrast, cells inoculated into medium containing both leucine and 2.5 μg of coumermycin per ml showed only slight inhibition of growth relative to controls grown in the absence of coumermycin (Fig. 1A). Thus, coumermycin suppressed the Leu+ phenotype of a top mutant carrying the leu-500 promoter mutation. Addition of leucine to the culture with coumermycin but without leucine allowed these cells to grow (Fig. 1A), confirming that inability to synthesize leucine was the defect which prevented growth. The long lag between the addition of leucine to this culture and the onset of growth was unexpected. Although we have not investigated the causes of this lag, one contributing factor may be that only 10 to 40% of the cells initially present were still viable by the time leucine was added in such experiments (data not shown).

The growth of this leu-500 top strain was dependent on added leucine at concentrations of approximately 2.5 to 3.5 μg of coumermycin per ml; higher coumermycin concentrations severely inhibited growth even in the presence of leucine (data not shown). Low concentrations of coumermycin had the expected effect on intracellular levels of supercoiling activity in this strain (Fig. 1B): pBR322 DNA isolated from cells grown in the presence of 2.5 μg of coumermycin per ml (lane 2) had considerable amounts of DNA that was less negatively supercoiled than a control isolated from cells grown in the absence of coumermycin (lanes 1 and 3). The result of the present study supports the hypothesis that suppression of the leu-500 promoter mutation by mutations in top is caused by increased DNA supercoiling: low levels of coumermycin which reduce intracellular levels of supercoiling activity cause growth of a leu-500 top mutant to become dependent on added leucine. DNA sequence information is also consistent with this hypothesis. The leu-500 mutation consists of the substitution of a G·C for an A·T base pair in the −10 region of the leucine promoter (8). This is the region in E. coli promoters which is unwound during initiation of transcription (23). Thus, the increased G+C content of the mutant −10 region might interfere with unwinding of the region by RNA polymerase. Increased negative supercoiling, on the other hand, facilitates unwinding of the DNA helix (9) and could thereby restore activity to the mutant promoter.

We thank Janice Zengel and Lasse Lindahl for valuable discussions and Julie Carlson for excellent secretarial assistance.

This work was supported by Public Health Service grants GM32005 and GM24320 from the National Institutes of Health.
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