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Antagonism of the B Subunit of DNA Gyrase Eliminates Plasmids pBR322 and pMG110 from *Escherichia coli*

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The constructed plasmid pBR322 and the native plasmid pMG110 were eliminated (cured) from growing *Escherichia coli* cells by the antagonism of the B subunit of the bacterial enzyme DNA gyrase. The antagonism may be by the growth of cells (i) at semipermissive temperatures in a bacterial mutant containing a thermolabile gyrase B subunit or (ii) at semipermissive concentrations of coumermycin A1, an antibiotic that specifically inhibits the B subunit of DNA gyrase. The kinetics of plasmid elimination indicate that plasmid loss occurs too rapidly to be explained solely by the faster growth of plasmid-free bacteria and, therefore, represents interference with plasmid maintenance.

A variety of chemical agents are capable of eliminating (curing) certain plasmids from their bacterial hosts. Such agents include acridine orange (1, 21, 23, 51, 61), acriflavine (19, 20, 41, 60), ethidium bromide (6), chloroquine (17), and sodium dodecyl sulfate (57). Many of these compounds are more effective in eliminating the sex factor F than in removing antibiotic resistance plasmids (R-plasmids) from enteric bacteria (59). Similarly, the antibiotic rifampin is capable of readily eliminating F' *lac* from *Escherichia coli* (2, 47), but causes a loss of R-plasmids only to a limited extent (31).

In contrast, the antibiotic novobiocin eliminated 7 of 13 R-plasmids and an F-plasmid from growing bacteria (40). The antibacterial action of novobiocin and structurally related compounds, such as coumermycin A1, results from the inhibition of the B subunit of the bacterial enzyme DNA gyrase (13). Although it is tempting to relate the plasmid-curing capacity of these drugs to their ability to inhibit DNA gyrase in vitro, it is possible that plasmid elimination reflects an action on a target unrelated to the gyrase. Recently, Taylor and Levine reported that the elimination of a mutant R-plasmid by novobiocin involves *E. coli* DNA gyrase (56). Subsequently, Danilevskaya and Gragerov showed that coumermycin A1 induces a loss of the ColE1-related, constructed plasmids pBR322 and pMB9 and that for pMB9 *E. coli* DNA gyrase is involved (8).

We now present experiments extending these observations on plasmid elimination and show (i) that pBR322 and the native plasmid pMG110 are cured from *E. coli* with a thermolabile DNA gyrase at temperatures semipermissive for growth, establishing independently of chemical inhibitors the requirement of a functioning gyrase B subunit for plasmid maintenance; (ii) that elimination of these same two plasmids by coumermycin A1 involves the antagonism of *E. coli* DNA gyrase; and (iii) that in kinetic experiments plasmid loss occurs too rapidly to be explained solely by the faster growth of plasmid-free bacteria and, therefore, represents interference with plasmid maintenance.

MATERIALS AND METHODS

Chemicals. Coumermycin A1 was a gift of W. Minor and K. Price, Bristol Laboratories (Syracuse, N.Y.). Cycloserine, a gift of R. Moellering and G. Eliopoulos, was made by Eli Lilly & Co. (Indianapolis, Ind.). Bromocresol purple (sodium salt) was from J. T. Baker Chemical Co. (Phillipsburg, N.J.); penicillin G potassium and chloramphenicol were from Parke, Davis & Co. (Morris Plains, N.J.); ampicillin sodium was from Bristol Laboratories (Syracuse, N.Y.); and tetracycline hydrochloride was from Pfizer Inc. (New York, N.Y.).

Bacterial strains and plasmids. *E. coli* N4177 [*galK2 gyrB41*(Ts)] and N99 (*galK2*) are isogenic strains, kindly given to us by M. Gellert, that differ only in their *gyrB* locus: N4177 carries a *gyrB* mutation (nitrosoguanidine induced in another strain and P1 transduced into N99) that results in coumermycin A1 resistance at 30°C or below and blocks bacterial growth at elevated temperatures; N99 (*gyrB*$^+$) is coumermycin A1 susceptible and grows well at 42°C. In vitro, the DNA gyrase subunit B purified from N4177 and assayed for supercoiling activity at 1.4 mM ATP is resistant to 10 μg of coumermycin A1 per ml and has less than 5% activity detectable at 42°C relative to 25°C; the N99 gyrase B subunit is inhibited by 1 μg of coumermycin A1 per ml and has equivalent activities at 42 and 25°C (M. Gellert, personal communication). The coumermycin A1-resistant and temperature-sensitive phenotypes of N4177 probably reside in *gyrB*, because spontaneous mutation to temperature insensi-

tivity is associated with reversion to coumermycin A1 susceptibility (D. Hooper, unpublished data).

pBR322, also given to us by M. Gellert, is a small, multicopy, nontransmissible, constructed plasmid that carries ampicillin and tetracycline resistance genes and has a ColE1-related replication region (5, 55). pBR322 was transformed into N4177 and N99 by the method of Lederberg and Cohen (37). Many of our experiments used bacterial strains carrying a circular dimer of pBR322 (determined by agarose gel electrophoresis and electron microscopy), which arose spontaneously and was stably maintained in recombination-proficient bacteria, as has been observed by others (3, 46). pBR322 dimers appear to be eliminated to a greater extent than monomers with the antagonism of the B subunit of DNA gyrase (J. Wolfson, D. Hooper, M. Swartz, unpublished data), possibly because dimer copy number may be lower than that of monomers (22).

Plasmid pMG110 was found in a multiply resistant *E. coli* clinical isolate, from the Bacteriology Laboratory of the Massachusetts General Hospital (kindly provided by L. Kunz and R. Moellering). pMG110 has a molecular mass of approximately 170 megadaltons, is conjugative, and codes for resistance to ampicillin, chloramphenicol, gentamicin, mercury, tellurite, tetracycline, and tobramycin. pMG110 belongs to the recently identified incompatibility group H II (D. Bradley, V. Hughes, M. Winters, D. Hooper, and M. Swartz, unpublished data).

Media and growth conditions. For the growth of bacteria in liquid cultures, the supplemented minimal medium of Kreuzer and Cozzarelli (46 mM K_2HPO_4, 23 mM KH_2PO_4, 8 mM $(NH_4)_2SO_4$, 0.4 mM $MgSO_4$, 6 mM $FeCl_3$, 1 mM sodium citrate, thiamine [1 μg/ml], glucose [5 mg/ml], thymine [5 μg/ml], uracil [5 μg/ml], and Casamino Acids [500 μg/ml]; 32) was used. A 10- or 20-ml amount of medium per 125-ml Erlenmeyer flask was shaken 100 times per min in a temperature-controlled, rotary-platform water bath. Inocula for experiments involving plasmid-containing bacteria were from overnight stationary cultures grown at 30°C in supplemented minimal medium containing tetracycline (20 μg/ml) and contained less than 1% plasmid-free bacteria. For determining titers, bacteria were diluted in 0.9% NaCl, plated on tryptone-yeast extract (TYE) agar ([per liter of water] 10 g of tryptone [Difco Laboratories, Detroit, Mich.], 5 g of yeast extract, 8 g of NaCl, 15 g of agar), and incubated at 30°C. In experiments testing the elimination of pBR322 dimer or pMG110 from N4177 and N99 at different temperatures, bacteria were inoculated from overnight cultures at 10^3 colony-forming units (CFU) per ml, incubated for 2 h at 25°C with agitation, transferred to water baths set at the indicated temperatures, and incubated with agitation for 18 h, at which time each culture was diluted and plated on TYE agar. After growth at 30°C, colonies were counted and scored for the presence of the plasmids.

Assay for plasmid presence or absence. The presence of plasmids in bacterial colonies was assayed with filter paper impregnated with penicillin and a pH-indicator dye ("test paper"). Bacteria containing certain beta-lactamases, including those specified by pBR322 and pMG110, turn test paper dye from green to yellow, presumably by the release of protons with enzymatic cleavage of penicillin. In the assay, test paper is touched for 10 s to a TYE agar surface containing colonies, which are partially transferred to the paper; when the paper dries, colonies are scored as plasmid containing (yellow), plasmid free (purple), or mixed (part yellow, part purple). Mixed colonies occasionally are seen and are counted as uncured, because at least some bacteria in the colony retain plasmids. Disparities between the results of test paper and replica-plating for individual colonies were assessed under all experimental conditions and were 0.36% (2/550). Test papers were made in the following way. Bromocresol purple (100 mg/ml, in water) was diluted 1/10 into a 20% aqueous solution of penicillin G. Whatman no. 2 filter paper was dipped into the mixture, allowed to dry, cut into a convenient size (usually 1 by 3 cm), and stored in scintillation vials in a desiccator jar at either 4°C (papers stable for weeks) or −20°C (papers stable for at least 12 months). The test paper assay is a modification of the method of Slack et al. (48a) for detection of β-lactamase production in limited numbers of clinical isolates of *Haemophilus influenzae*.

For replica-plating to detect the presence of pBR322, the template plate contained TYE agar, and velvet replicas were transferred successively to three MacConkey agar plates containing tetracycline (50 μg/ml), ampicillin (25 μg/ml), and no antibiotic; for the detection of pMG110, resistance to chloramphenicol (50 μg/ml) was additionally scored.

Statistical methods. For the analysis of the concentration dependence of coumermycin A1-induced plasmid elimination from N4177(pBR322) and N99(pBR322), the data were fitted with a square root transformation to straight lines with the same slope; the significance of the difference in the *y*-intercept of these two lines, reflecting the concentration difference effective in equivalent plasmid elimination in the two strains, was then assessed by Student's *t* test.

RESULTS

Plasmid elimination from bacteria temperature-sensitive in the B subunit of DNA gyrase. N4177 [*gyrB*(Ts)] carrying pBR322 was grown at permissive (25°C) and semipermissive (36.5°C) temperatures in liquid medium, titers were determined, and the colonies were assayed for the presence of plasmids. The elimination of pBR322 after 14 generations of growth at 36.5°C was 92%, but only 1% after 16 generations of growth at 25°C. In contrast, a 2% loss of pBR322 from strain N99 (*gyrB*$^+$) was seen at both temperatures, indicating that thermolability was not intrinsic to the plasmid DNA molecule. We interpret these data as evidence that pBR322 requires a functional gyrase B subunit to be maintained in a population of growing bacteria.

Similar results were obtained for plasmid pMG110: elimination from strain N4177 was 6% at 30°C, 46% at 35°C, and >99% at 37°C. Curing from N99 was <1% at all three temperatures.

To determine the rate of plasmid elimination occurring at permissive and semipermissive temperatures and the growth rates of plasmid-containing and plasmid-free cells, we studied the

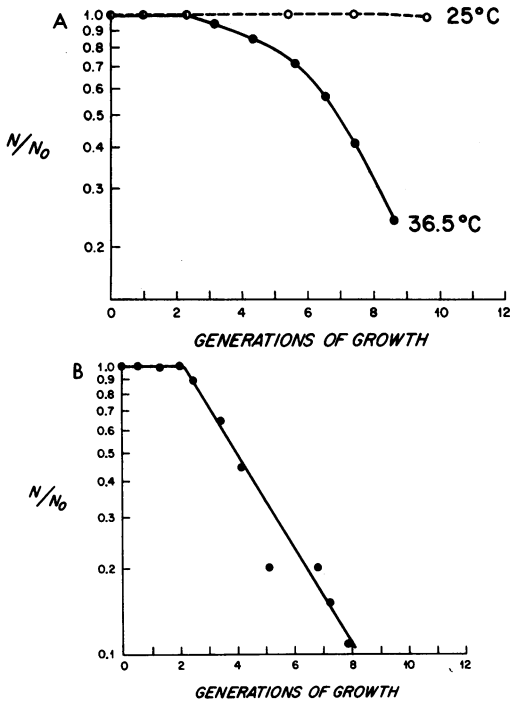

FIG. 1. (A) Kinetics of elimination of pBR322 (dimer) from N4177 [*gyrB*(Ts)] at 25 and 36.5°C. N4177(pBR322 dimer) was diluted to 1.4×10^3 CFU/ml in 20 ml of medium in each of two flasks and incubated for 2 h at 25°C with agitation; one flask was then transferred to 36.5°C, and bacteria from each flask were plated hourly. An additional culture of N4177 without plasmid was incubated at 36.5°C and plated at similar intervals (data not shown). Plasmid loss from N4177(pBR322 dimer) at 25°C (○) and at 36.5°C (●) versus generations of bacterial growth. N = plasmid-containing cells; N_0 = total number of cells. For each point, at least 50 but usually greater than 150 colonies were scored. (B) Kinetics of elimination of pMG110 from N4177 [*gyrB*(Ts)] at 36°C. The experiment was performed as described for N4177(pBR322 dimer). The initial inoculum of N4177(pMG110) was 4.2×10^3 CFU/ml.

time course of plasmid elimination. In N4177(pBR322), plasmid loss was first detected after 3 generations of growth at 36.5°C, and the rate of elimination appeared to increase with continued bacterial multiplication to 9 generations (Fig. 1A); pBR322 was stable in N4177 during 10 generations of growth at 25°C. The growth rate of N4177 without plasmids (47 min/generation) was somewhat more rapid than that of N4177(pBR322) (54 min/generation) over the course of the experiment (data not shown). From these data, it is possible to estimate the contribution of growth rate differences to the magnitude of plasmid elimination observed. Early in the experiment, when plasmid loss is first detectable, the cells grew from 3.4×10^4 CFU/ml to 1.4×10^5 CFU/ml over 120 min; during this time, the fraction of cells without plasmids increased from no more than 0.01 to 0.15 (from 2.3 to 4.3 generations of growth, Fig. 1A). Thus, the initial concentration of plasmid-free cells, $0.01 \times 3.4 \times 10^4 = 3.4 \times 10^2$ CFU/ml, would reach 2.0×10^3 CFU/ml in 120 min (120 min/[47 min/generation] = 2.55 generations; $3.4 \times 10^2 \times 2^{2.55} = 2.0 \times 10^3$ CFU/ml). The observed number of plasmid-free cells at the later time, however, is $0.15 \times 1.4 \times 10^5 = 2.1 \times 10^4$ CFU/ml, over 10-fold higher. We interpret these estimates as evidence that plasmid-free cells appearing early in curing are derived not only from the growth of preexisting plasmid-free cells, but also, to a greater extent, from the curing of plasmid-containing cells. In the above calculations, we assume that the growth rate of N4177 in a flask separate from N4177(pBR322) is the same as if the two strains were growing in the same flask.

Similar kinetic experiments were performed with N4177(pMG110) (Fig. 1B). Generation times for N4177(pMG110) and N4177 were 63 and 56 min, respectively (data not shown). Calculations like those above for pBR322 revealed that the faster growth of bacteria lacking pMG110 was able to account for the appearance of only 22% of the plasmid-free cells.

Plasmid elimination by coumermycin A1: involvement of DNA gyrase. Experiments investigating the involvement of *E. coli* DNA gyrase in plasmid elimination by coumermycin A1 were carried out with strains N4177 and N99 at 25°C and 30°C, temperatures at which N4177 grows well and at which its *gyrB* mutation confers resistance to 12 μg of coumermycin A1 per ml. Cultures of N4177(pBR322) and N99(pBR322) were grown for 26 h at 25°C in the presence of increasing concentrations of coumermycin A1, titers were determined, and the colonies were scored for plasmid presence. N4177(pBR322) grew to a final titer of greater than 1×10^8 CFU/ml at all coumermycin A1 concentrations, whereas the growth of N99(pBR322) was retarded at higher drug concentrations (Fig. 2A), confirming the coumermycin A1 resistance of N4177. The concentration of coumermycin A1 producing equivalent levels of plasmid elimination was higher in N4177 by a factor of slightly less than two (Fig. 2B). This difference in drug concentration was significant ($P < 0.001$), and its magnitude was consistent with the difference in coumermycin A1 required to inhibit the multiplication of the two strains (data not shown). That pBR322 is eliminated from N4177 at drug concentrations that do not affect the final bacterial titer is perhaps surprising; however, this curing is occurring under conditions that likely are semipermissive with bacteria growing more slowly but still reaching the control cell titer

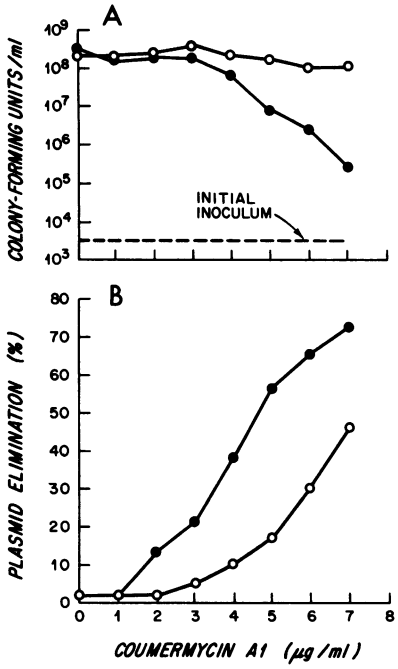

FIG. 2. Elimination of pBR322 (dimer) from N4177 (Cou^r) and N99 (Cou^s) by coumermycin A1 at 25°C. N4177(pBR322 dimer) and N99(pBR322 dimer) were inoculated at 2.0×10^3 and 5.9×10^3 CFU/ml, respectively, in 200 ml of medium, incubated at 25°C with agitation for 3 h, divided into 10-ml portions per flask with indicated concentrations of coumermycin A1, incubated with agitation at 25°C for an additional 24 h, and plated on TYE agar. CFU per milliliter (A) and plasmid elimination (B) after 24 h of growth at different concentrations of coumermycin A1 for N4177(pBR322 dimer) (○) and N99(pBR322 dimer) (●).

within 24 h. To confirm the difference in pBR322 stability in N4177 and N99 in the presence of coumermycin A1, the kinetics of plasmid elimination from both strains were determined at 30°C: the rate of plasmid elimination at 4.5 μg of coumermycin A1 per ml was more rapid from N99 than from N4177 (Fig. 3). Because N4177 and N99 differ only at the *gyrB* locus, we interpret these findings as evidence that the host gyrase B subunit is involved in coumermycin A1 curing of pBR322 from these bacterial strains.

To assess an alternative possibility that bacteria growing poorly under unfavorable conditions (such as the presence of near-inhibitory concentrations of an antibiotic) may lose plasmids nonspecifically, N4177(pBR322) and N99(pBR322) were grown in the presence of increasing concentrations of cycloserine, an inhibitor of bacterial cell wall synthesis. Growth at multiple concentrations of cycloserine (from 0 to 20 μg/ml), extending to those subinhibitory and fully inhibitory to bacterial multiplication, resulted in no plasmid elimination above a 2% spontaneous background loss from both strains (data not shown).

In additional kinetic experiments, the growth rates of N4177(pBR322) and N4177 were related to the rate of pBR322 elimination in the presence of 6 μg of coumermycin A1 per ml (data not shown). The growth curves of N4177(pBR322) and N4177 were both biphasic (data not shown), a reproducible finding suggesting possible cellular adaptations to growth in the presence of coumermycin A1. Using the later, more rapid growth rate values [N4177(pBR322) generation time = 70.5 min, N4177 generation time = 60 min], calculations like those presented in the preceding section showed that growth rate differences could account for the emergence of only 28% of the plasmid-free cells. Thus, the appearance of N4177 without pBR322 occurred too rapidly to be explained only by the faster growth of plasmid-free cells.

Experiments with N4177(pMG110) and N99(pMG110) growing in the presence of varied concentrations of coumermycin A1 revealed that pMG110 was eliminated only from N99 (Fig. 4). We interpret these results as evidence that coumermycin A1-induced elimination of pMG110 from N99, like that of pBR322, involves the drug antagonism of *E. coli* DNA gyrase. Failure to eliminate pMG110 from N4177 over a broad range of concentrations is further evidence that the antagonism of growth alone is insufficient to explain plasmid elimination by coumermycin A1. In these experiments,

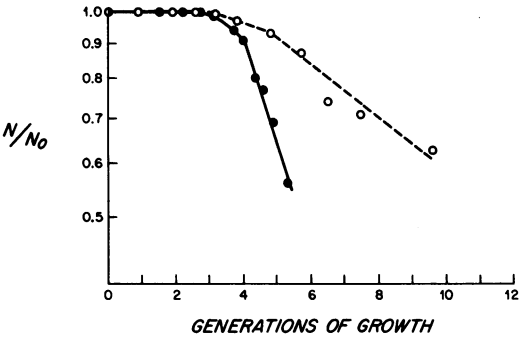

FIG. 3. Kinetics of elimination of pBR322 (dimer) from N4177 (Cou^r) and N99 (Cou^s) by coumermycin A1 (4.5 μg/ml) at 30°C. N4177(pBR322 dimer) and N99(pBR322 dimer) were inoculated at 4.5×10^3 and 2.7×10^3 CFU/ml, respectively, in 20 ml of medium and incubated at 30°C with agitation for 2 h. Coumermycin A1 was added to 4.5 μg/ml (at a titer designated 0 generations), and incubation was continued, with aliquots plated at hourly intervals. N4177(pBR322 dimer) (○) and N99(pBR322 dimer) (●) versus generations of bacterial growth.

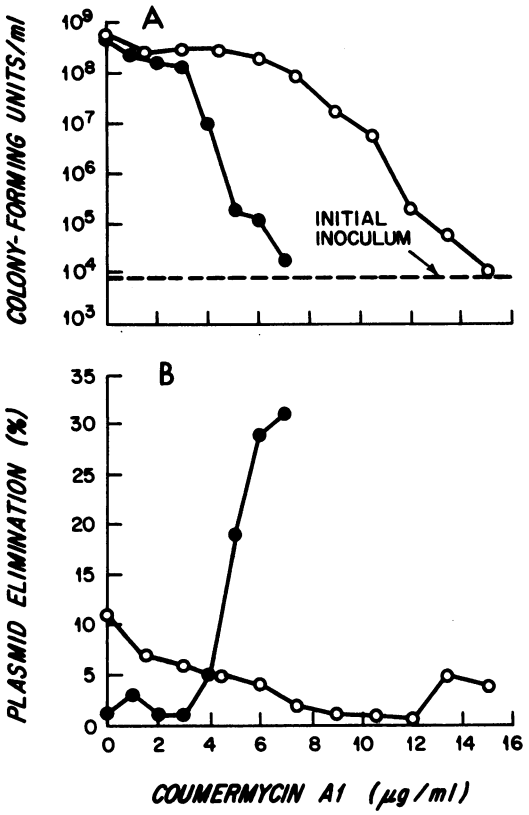

FIG. 4. Elimination of pMG110 from N4177 (Cou^r) and N99 (Cou^s) by coumermycin A1 at 25°C. The experiment was performed as described in the legend to Fig. 2. Initial inocula of N4177(pMG110) and N99(pMG110) were 3.9×10^3 and 3.0×10^3 CFU/ml, respectively. (A) CFU per milliliter after 24 h of growth. (B) Plasmid elimination at different concentrations of coumermycin A1 for N4177(pMG110) (○) and N99(pMG110) (●).

in the absence of coumermycin A1, the spontaneous loss of pMG110 was greater from N4177 than from N99, as described earlier in this manuscript, confirming that a mutation in the gyrase B subunit can alter the stability of pMG110.

Because coumermycin A1-induced elimination of pMG110 from N99 occurs at drug concentrations very near those inhibiting bacterial multiplication, it was not possible to generate data on the kinetics of pMG110 loss from N99 adequate to assess the contribution of N99(pMG110) and N99 growth rate differences.

DISCUSSION

Prior studies by two groups of investigators have documented the involvement of bacterial DNA gyrase in plasmid elimination by novobiocin and coumermycin A1. Taylor and Levine (56) isolated a temperature-sensitive mutant (pDT4) of an incompatibility group H2 plasmid which, in contrast to the parental plasmid, was eliminated from *E. coli* by novobiocin at permissive temperatures. Danilevskaya and Gragerov (8) showed that subinhibitory concentrations of coumermycin A1 eliminated the constructed ColE1-related plasmids pBR322, pMB9, and pOD162. Neither pDT4 nor pMB9 was eliminated from *E. coli* strains containing a *gyrB* mutation determining resistance to coumermycin A1, thus establishing the involvement of bacterial DNA gyrase in plasmid elimination by novobiocin and coumermycin A1 for pDT4 (56) and pMB9 (8), respectively. Our experiments extend these findings for coumermycin A1 to include pBR322 and the first wild-type plasmid, pMG110, confirming that DNA gyrase is a target of coumermycin A1 in producing plasmid elimination. Additionally, our kinetic studies identified two components contributing to the emergence of plasmid-free cells during the antagonism of gyrase: loss of plasmids from plasmid-containing bacteria and faster growth of plasmid-free cells. The latter component is not unexpected. Even under noncompromised conditions of growth, plasmid-containing cells may multiply more slowly than plasmid-free cells (14, 18, 29), and such a difference may be accentuated if replicating plasmid and bacterial DNA are competing for a limiting intracellular pool of functioning gyrase.

Because the use of chemical inhibitors in studies of in vivo functions leaves uncertainties regarding drug permeability, drug metabolism, and secondary targets, it is important to assess, if possible, the role of an enzyme in vivo in the absence of inhibitors. Our data with a temperature-sensitive *gyrB* mutant demonstrate for the first time in the absence of novobiocin and coumermycin A1 the requirement for a functioning gyrase B subunit for the maintenance of plasmids in vivo.

In considering possible mechanisms for plasmid elimination with gyrase antagonism, it is worthwhile reviewing briefly the structure and some activities of DNA gyrase. The enzyme has four subunits: two "A," antagonized by nalidixic acid and oxolinic acid (11, 52), and two "B," inhibited by novobiocin, coumermycin, and structurally related compounds. In vitro, gyrase catalyzes a variety of reactions (7, 10), including the introduction of negative superhelical twists into covalently closed double-stranded DNA circles (12) (like plasmid DNA molecules) and separation (decatenation) and rejoining (catenation) of two DNA circles interlocked like links in a chain (25, 33). In vivo, gyrase maintains negative superhelical twists in intracellular DNA

circles (12, 44) and is necessary for DNA replication, the transcription of certain operons, DNA repair, and recombination (7, 10).

Because the elimination of plasmids by the antagonism of gyrase occurs during continued bacterial multiplication, a process essential for plasmid maintenance appears to be more susceptible to the inhibition of DNA gyrase than is bacterial multiplication. Possible sites for such a selective interference in the plasmid life cycle are many, but five merit specific mention. (i) Gyrase antagonism might cause the loss of negative superhelical twists needed for the initiation of plasmid DNA replication (16, 27, 44, 45). (ii) The inhibition of gyrase might impair the decatenation of two interlocked, covalently closed monomeric DNA circles, the product of a round of plasmid replication via the Cairns form (9, 15, 24, 25, 28, 34, 43, 48, 53, 54); this failure of decatenation might cause aberrant segregation (33, 42). (iii) The antagonism of gyrase might result in the defective removal of positive superhelical twists that might accumulate ahead of and slow the progression of the growing point in replicating Cairns forms (27). (iv) Gyrase inhibition might result in a failure to repair plasmid DNA molecules effectively, leading to plasmid destruction. Such destruction has been reported for ColE1 DNA after treatment with chloramphenicol and coumermycin A1 (8). Not addressed in that study (8), however, was the contribution of chloramphenicol treatment, which results in the presence of RNA bases in mature ColE1 DNA (4) that might then be aberrantly repaired. Finally (v), selective effects of DNA gyrase inhibition on plasmid maintenance might result directly from differing affinities of gyrase for binding sites on plasmid and bacterial DNA; DNA gyrase has preferred binding sequences on plasmid molecules in vitro (7, 10) and, presumably, does also on plasmid and bacterial DNA in vivo (50). Of these mechanisms for plasmid elimination by gyrase antagonism, we consider the inhibition of the initiation of DNA replication and aberrant decatenation as leading possibilities. It seems not unlikely that different mechanisms may operate in the elimination of different plasmids.

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LITERATURE CITED

1. **Bastarrachea, F., and N. S. Willetts.** 1968. The elimination by acridine orange of F30 from recombination-deficient strains of *Escherichia coli* K12. Genetics **59**:153–166.
2. **Bazzicalupo, P., and G. P. Tocchini-Valentini.** 1972. Curing of an *Escherichia coli* episome by rifampin. Proc. Natl. Acad. Sci. U.S.A. **69**:298–300.
3. **Bedbrook, J. R., and F. M. Ausubel.** 1976. Recombination between bacterial plasmids leading to the formation of plasmid multimers. Cell **9**:707–716.
4. **Blair, D. G., D. J. Sherratt, D. B. Clewell, and D. R. Helinski.** 1972. Isolation of supercoiled colicinogenic factor E1 DNA sensitive to ribonuclease and alkali. Proc. Natl. Acad. Sci. U.S.A. **69**:2518–2522.
5. **Bolivar, F., R. L. Rodriguez, P. J. Green, M. C. Betlach, H. L. Heyneker, H. W. Boyer, J. H. Crosa, and S. Falkow.** 1977. Construction and characterization of new cloning vehicles. II. A multi-purpose cloning system. Gene **2**:95–113.
6. **Bouanchaud, D. H., M. R. Scavizzi, and Y. A. Chabbert.** 1969. Elimination by ethidium bromide of antibiotic resistance in enterobacteria and staphylococci. J. Gen. Microbiol. **54**:417–425.
7. **Cozzarelli, N. R.** 1980. DNA gyrase and the supercoiling of DNA. Science **207**:953–960.
8. **Danilevskaya, O. N., and A. I. Gragerov.** 1980. Curing of *Escherichia coli* K12 plasmids by coumermycin. Mol. Gen. Genet. **178**:233–235.
9. **Fuke, M., and J. Inselburg.** 1972. Electron microscopic studies of replicating and catenated colicin factor E1 DNA isolated from minicells. Proc. Natl. Acad. Sci. U.S.A. **69**:89–92.
10. **Gellert, M.** 1981. DNA topoisomerases. Annu. Rev. Biochem. **50**:879–910.
11. **Gellert, M., K. Mizuuchi, M. H. O'Dea, T. Itoh, and J. Tomizawa.** 1977. Nalidixic acid resistance: a second genetic character involved in DNA gyrase activity. Proc. Natl. Acad. Sci. U.S.A. **74**:4772–4776.
12. **Gellert, M., K. Mizuuchi, M. H. O'Dea, and H. A. Nash.** 1976. DNA gyrase: an enzyme that introduces superhelical turns into DNA. Proc. Natl. Acad. Sci. U.S.A. **73**:3872–3876.
13. **Gellert, M., M. H. O'Dea, T. Itoh, and J. Tomizawa.** 1976. Novobiocin and coumermycin inhibit DNA supercoiling catalyzed by DNA gyrase. Proc. Natl. Acad. Sci. U.S.A. **73**:4474–4478.
14. **Godwin, D., and J. H. Slater.** 1979. The influence of the growth environment on the stability of a drug resistance plasmid in *Escherichia coli* K12. J. Gen. Microbiol. **111**:201–210.
15. **Goebel, W.** 1971. Formation of complex ColE1 DNA by replication. Biochim. Biophys. Acta **232**:32–42.
16. **Gustafsson, P., H. Dreisig, S. Molin, K. Nordstrom, and B. E. Uhlin.** 1979. DNA replication control: studies of plasmid R1. Cold Spring Harbor Symp Quant. Biol. **43**:419–425.
17. **Hahn, F. E., and J. Ciak.** 1976. Elimination of resistance determinants from R-factor R1 by intercalative compounds. Antimicrob. Agents Chemother. **9**:77–80.
18. **Helling, R. B., T. Kinney, and J. Adams.** 1981. The maintenance of plasmid-containing organisms in populations of *Escherichia coli*. J. Gen. Microbiol. **123**:129–141.
19. **Hirota, Y.** 1960. The effect of acridine dyes on mating type factors in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **46**:57–64.
20. **Hirota, Y., and T. Iijima.** 1957. Acriflavine as an effective agent for eliminating F-factor in *Escherichia coli* K-12. Nature (London) **180**:655–656.
21. **Hohn B., and D. Korn.** 1969. Cosegregation of a sex factor with the *Escherichia coli* chromosome during curing by acridine orange. J. Mol. Biol. **45**:385–395.
22. **Ike, Y., H. Hashimoto, and S. Mitsuhashi.** 1981. Regular segregation of composite plasmid Rms201. J. Bacteriol.

148:534–540.
23. **Inselburg, J.** 1971. R factor deoxyribonucleic acid in chromosomeless progeny of *Escherichia coli*. J. Bacteriol. 105:620–628.
24. **Inselburg, J., and M. Fuke.** 1971. Isolation of catenated and replicating DNA molecules of colicin factor E1 from minicells. Proc. Natl. Acad. Sci. U.S.A. 68:2839–2942.
25. **Inselburg, J. W.** 1973. Formation of catenated colicin factor E1 deoxyribonucleic acid molecules in a recombination-deficient strain of *Escherichia coli*. J. Bacteriol. 113:1084–1085.
26. **Inuzuka, M., and D. R. Helinski.** 1978. Requirement of a plasmid-encoded protein for replication *in vitro* of plasmid R6K. Proc. Natl. Acad. Sci. U.S.A. 75:5381–5385.
27. **Itoh, T., and J. Tomizawa.** 1979. Initiation of replication of plasmid ColE1 DNA by RNA polymerase, ribonuclease H, and DNA polymerase I. Cold Spring Harbor Symp. Quant. Biol. 43:409–417.
28. **Jaenisch, R., and A. J. Levine.** 1973. DNA replication of SV40-infected cells. VII. Formation of SV40 catenated and circular dimers. J. Mol. Biol. 73:199–212.
29. **Jones, I. M., S. B. Primrose, A. Robinson, and D. C. Ellwood.** 1980. Maintenance of some ColE1-type plasmids in chemostat culture. Mol. Gen. Genet. 180:579–584.
30. **Kolter, R., M. Inuzuka, and D. R. Helinski.** 1978. Trans-complementation-dependent replication of a low molecular weight origin fragment from plasmid R6K. Cell 15:1199–1208.
31. **Kremery, V., and J. Janouskova.** 1971. Effect of rifampin on stability and transfer of R-factors. Z. Allg. Mikrobiol. 11:97–101.
32. **Kreuzer, K. N., and N. R. Cozzarelli.** 1979. *Escherichia coli* mutants thermosensitive for deoxyribonucleic acid gyrase subunit A: effects on deoxyribonucleic acid replication, transcription, and bacteriophage growth. J. Bacteriol. 140:424–435.
33. **Kreuzer, K. N., and N. R. Cozzarelli.** 1980. Formation and resolution of DNA catenanes by DNA gyrase. Cell 20:245–254.
34. **Kupersztoch, Y. M., and D. R. Helinski.** 1973. A catenated DNA molecule as an intermediate in the replication of the resistance transfer factor R6K in *Escherichia coli*. Biochem. Biophys. Res. Commun. 54:1451–1459.
35. **Lanka, E., and P. T. Barth.** 1981. Plasmid RP4 specifies a deoxyribonucleic primase involved in its conjugal transfer and maintenance. J. Bacteriol. 148:769–781.
36. **Lanka, E., E. Scherzinger, E. Gunther, and H. Schuster.** 1979. A DNA primase specified by I-like plasmids. Proc. Natl. Acad. Sci. U.S.A. 76:3632–3636.
37. **Lederberg, E. M., and S. N. Cohen.** 1974. Transformation of *Salmonella typhimurium* by plasmid deoxyribonucleic acid. J. Bacteriol. 119:1072–1074.
38. **Liu, L., C. Liu, and B. M. Alberts.** 1980. Type II DNA topoisomerases: enzymes that can unknot a topologically knotted DNA molecule via a reversible double-strand break. Cell 19:697–707.
39. **Marini, J. C., K. G. Miller, and P. T. Englund.** 1980. Decatenation of kinetoplast DNA by topoisomerases. J. Biol. Chem. 255:4976–4979.
40. **McHugh, G. L., and M. N. Swartz.** 1977. Elimination of plasmids from several bacterial species by novobiocin. Antimicrob. Agents Chemother. 12:423–426.
41. **Mitsuhashi, S., K. Harada, and M. Kameda.** 1961. Elimination of transmissible drug-resistance by treatment with acriflavin. Nature (London) 189:947.
42. **Mizuuchi, K., L. M. Fisher, M. H. O'Dea, and M. Gellert.** 1980. DNA gyrase action involves the introduction of transient double-strand breaks. Proc. Natl. Acad. Sci. U.S.A. 77:1847–1851.
43. **Novick, R. P., K. Smith, R. J. Sheehy, and E. Murphy.** 1973. A catenated intermediate in plasmid replication. Biochem. Biophys. Res. Commun. 54:1460–1469.
44. **Ogasawara, N., M. Seiki, and H. Yoshikawa.** 1981. Initiation of DNA replication in *Bacillus subtilis*. V. Role of DNA gyrase and superhelical structure in initiation. Mol. Gen. Genet. 181:332–337.
45. **Orr, E., and W. L. Staudenbauer.** 1981. An *Escherichia coli* mutant thermosensitive in the B subunit of DNA gyrase: effect on the structure and replication of the colicin E1 plasmid *in vitro*. Mol. Gen. Genet. 181:52–56.
46. **Potter, H., and D. Dressler.** 1977. On the mechanism of genetic recombination: the maturation of recombination intermediates. Proc. Natl. Acad. Sci. U.S.A. 74:4168–4172.
47. **Riva, S., A. Fietta, M. Berti, L. G. Silvestri, and E. Romero.** 1973. Relationships between curing of the F episome by rifampin and by acridine orange in *Escherichia coli*. Antimicrob. Agents Chemother. 3:456–462.
48. **Sakikibara, Y., K. Suzuki, and J. Tomizawa.** 1976. Formation of catenated molecules by replication of colicin E1 plasmid DNA in cell extracts. J. Mol. Biol. 108:569–582.
48a. **Slack, M. P., D. B. Wheldon, and D. C. Turk.** 1977. A rapid test for beta-lactamase production by *Hemophilus influenzae*. Lancet ii:906.
49. **Smith, G. R.** 1981. DNA supercoiling: another level for regulating gene expression. Cell 24:599–600.
50. **Snyder, M., and K. Drlica.** 1979. DNA gyrase on the bacterial chromosome: DNA cleavage induced by oxolinic acid. J. Mol. Biol. 131:287–302.
51. **Stouthamer, A. H., P. G. Deltaan, and E. J. Bulten.** 1963. Kinetics of F-curing by acridine orange in relation to the number of F-particles in *Escherichia coli*. Gen. Res. 4:305–317.
52. **Sugino, A., C. L. Peebles, K. N. Kreuzer, and N. R. Cozzarelli.** 1977. Mechanism of action of nalidixic acid: purification of *Escherichia coli* nalA gene product and its relationship to DNA gyrase and a novel nicking-closing enzyme. Proc. Natl. Acad. Sci. U.S.A. 74:4767–4771.
53. **Sundin, O., and A. Varshavsky.** 1980. Terminal stages of SV40 DNA replication proceed via multiply intertwined catenated dimers. Cell 21:103–114.
54. **Sundin, O., and A. Varshavsky.** 1981. Arrest of segregation leads to accumulation of highly intertwined catenated dimers: dissection of the final stages of SV40 DNA replication. Cell 25:659–669.
55. **Sutcliffe, J. G.** 1979. Complete nucleotide sequence of the *Escherichia coli* plasmid pBR322. Cold Spring Harbor Symp. Quant. Biol. 43:77–90.
56. **Taylor, D. E., and J. G. Levine.** 1979. Characterization of a plasmid mutation affecting maintenance, transfer, and elimination by novobiocin. Mol. Gen. Genet. 174:127–133.
57. **Tomoeda, M., M. Inuzuka, N. Kubo, and S. Nakamura.** 1968. Effective elimination of drug resistance in sex factors in *Escherichia coli* by sodium dodecyl sulfate. J. Bacteriol. 95:1078–1089.
58. **von Wright, A., and B. A. Bridges.** 1981. Effect of *gyrB*-mediated changes in chromosome structure on killing of *Escherichia coli* by ultraviolet light: experiments with strains differing in deoxyribonucleic acid repair capacity. J. Bacteriol. 146:18–23.
59. **Watanabe, T.** 1969. Transferable drug resistance: the nature of the problem, p. 92. *In* G. E. W. Wolstenholme and M. O'Connor (ed.), Bacterial episomes and plasmids. Little, Brown & Co., Boston.
60. **Watanabe, T., and T. Fukasawa.** 1961. Episome-mediated transfer of drug resistance in *Enterobacteriaceae*. II. Elimination of resistance factors with acridine dyes. J. Bacteriol. 81:679–683.
61. **Wechsler, J., and B. C. Kline.** 1980. Mutation and identification of the F plasmid locus determining resistance to acridine orange curing. Plasmid 4:276–280.