Involvement of DNA Gyrase in Bacteriophages T7 Growth

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We have found that the burst size of bacteriophage T7 was decreased in two Escherichia coli temperature-sensitive gyrA mutants incubated at the restrictive temperature. This reduction in burst size indicates that gyrase may be required for T7 growth.

In bacterial cells, closed circular DNA is under superhelical tension, and it appears that the degree of supercoiling is important for a variety of processes such as replication, transcription, and recombination (reviewed in references 2, 4, 6, and 17). Although cells infected with the linear DNA bacteriophage T4 contain phage DNA that may be under superhelical tension (16), it is not known whether topological constraints and superhelicity are general properties of linear DNA molecules. Studies of supercoiling during infection with the linear DNA phage T7 have been inconclusive. Inhibition of phage growth by antibiotics that block the host cell gyrase is consistent with the involvement of this enzyme in T7 growth. Gyrase is a topoisomerase that can introduce superhelical tension into DNA (7); the enzyme is composed of two subunit types, encoded by the gyrA and gyrB genes, and each subunit can be selectively inhibited by antibiotics or mutations (2, 6, 17). Inhibitors of either the Gyrb protein (nalidixic acid) (1, 10) or the GyrB protein (coumermycin A1) (3, 9) prevent T7 growth. In contrast, gyrase is not required for T7 DNA synthesis in reconstituted replication systems (11, 14, 15). These results can be reconciled if T7 DNA is topologically constrained in vivo but not in vitro.

Kreuzer and Cozzarelli (10) have reported that a temperature-sensitive gyrA mutation and nalidixic acid differ in their effects on T7 growth. Whereas addition of nalidixic acid to a gyrA42 mutant incubated at the permissive temperature inhibits T7 growth, incubation at the restrictive temperature without nalidixic acid causes only a 37% decrease in T7 burst size. These results can be explained in two ways: either gyrase is not required for T7 growth, or gyrA43 is a leaky mutation with respect to T7 growth (the mutation is clearly not leaky with respect to phage dX174 growth [10]). The observation that incubation at the restrictive temperature eliminates inhibition of phage T7 growth by nalidixic acid led Kreuzer and Cozzarelli to conclude that T7 does not require gyrase activity for growth. Presumably, inhibition of T7 growth by nalidixic acid at the permissive temperature arises from an altered reaction intermediate formed between nalidixic acid, its target protein, and DNA (13); at the restrictive temperature, a thermally altered protein would not be able to form this complex. However, complex formation may not be a measure of gyrase activity. Furthermore, chromosomal DNA replication remains sensitive to nalidixic acid at the restrictive temperature, making interpretation of these nalidixic acid studies difficult (10).

Because the possibility that gyrA43 is a leaky mutation still exists, we measured T7 burst sizes in two gyrB(Ts) mutants, Escherichia coli EC1510 gyrB41 (5) and E. coli EC1512 gyrB402 (5), to determine whether T7 growth requires gyrase. Incubation of either the gyrB41 or gyrB402 mutant at 43°C reduces chromosomal DNA supercoiling by more than 75% compared with that in mutants grown at 30°C (18). The mutants differ in two respects. First, cooling the cells from 43 to 0°C over a 3- to 4-min period before lysis allows the superhelical density to return to the level observed at 30°C in the gyrB41 mutant but not in the gyrB402 mutant (18). Second, the gyrB402 mutation inhibits the elongation step of replication at the restrictive temperature, but the gyrB41 mutation does not (5). These observations support the idea that the GyrB41 protein is less thermolabile than the GyrB402 protein. One-step growth experiments were performed by using a procedure similar to that of Kreuzer and Cozzarelli (10) at multiplicities of infection less than 0.2 and adsorption times of 4 to 7 min. Phage were added to parallel cultures that had been preincubated at 43°C for 0, 10, or 60 min before phage addition. The adsorption efficiency varied between 31 and 85%.

Results from representative one-step growth experiments are shown in Fig. 1. Shifting the wild-type strain (EC6 [5]) from 30 to 43°C resulted in a 70% increase in burst size over that observed at 30°C (Fig. 1A). By comparison, the burst size in strain EC1512 (gyrB402) was reduced by 75% when phage were added after a 10-min preincubation at 43°C and by 90% when phage were added after a 60-min preincubation at 43°C (Fig. 1B). The second mutant, strain EC1510 (gyrB41), also had reduced burst sizes at 43°C compared with those at 30°C but only after 60 min of preincubation at 43°C (Table 1).

These results indicate that the gyrB gene product is required for T7 growth; burst size was reduced at the restrictive temperature in both gyrB (Ts) mutants. After 10 min of preincubation at 43°C, T7 growth was inhibited to a greater extent in strain EC1512 (gyrB402) than in strain EC1510 (gyrB41). Thus, it appears that gyrase activity is lost more rapidly in the gyrB402 mutant than in the gyrB41 mutant. In both mutants, incomplete inhibition of phage growth was observed after a 10-min preincubation at 43°C. This inhibition may be due to a slow loss of gyrase activity arising from protein thermolability or decreased gyrase production or both. The inhibition of phage growth may also be due to indirect effects such as changes in the patterns of gene expression (3). The two mutations inhibited T7 growth at different rates, but both reduced supercoiling by more than 75%; this suggests that the indirect effects of changes in chromosomal supercoiling may not be major factors in the inhibition of T7 growth.

Since the GyrB protein alone does not alter DNA topology in vitro (8), it is likely that gyrase is involved in T7 growth. However, as pointed out above, Kreuzer and Cozzarelli (10) argue against involvement of the GyrA protein. We repeated their T7 growth experiments with E. coli KNU453 (gyrA43)

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and the isogenic wild-type E. coli HF4704. Our results were similar to those reported by Kreuzer and Cozzarelli (10); burst size decreased by approximately 56% when the results were corrected for the effect of temperature on strain HF4704 (Table 1). Thus, the gyrA43 mutant, like the gyrB41 mutant, appears to retain sufficient activity at the restrictive temperature to support some T7 growth. At the restrictive temperature the GyrA43 protein is probably altered enough to prevent nalidixic acid-induced inhibition of phage growth (10). We cannot explain the observation that adding nalidixic acid to a gyrA43 mutant at the restrictive temperature has no effect on T7 burst size but rapidly inhibits chromosomal replication (10).

Although we believe that gyrase activity is required for T7 growth, there are other possible interpretations of these results. For example, only the GyrB subunit of gyrase may be required for T7 growth, perhaps acting either alone or in conjunction with an unidentified protein to directly affect a process such as gene expression. It is unlikely that thermally inactivated GyrB protein inhibits T7 growth by forming a complex with DNA that prevents replication fork movement, because incubating strain EC1510 at the restrictive temperature inhibited T7 growth (Fig. 1C) but not the elongation phase of chromosome replication (5).

Gyrase can be envisioned as having two topological functions. First, it could reduce overwinding of the DNA helix which is thought to be introduced by migration of replication forks. Second, it could unwind the DNA helix to activate the DNA for processes involving strand separation (6). Both functions require that the substrate DNA be topologically constrained. Since unconstrained linear DNA would be able to relieve superhelical tension by rotation of the free DNA ends, the observations that gyrase is required for the growth of linear DNA phage T7 (1, 3, 9; this study) and that DNA is under negative torsional tension during infection with T4 (16) suggest that these genomes are topologically constrained in vivo. These results indicate that superhelical tension must be taken into account in studying the growth of linear DNA bacteriophages.

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**LITERATURE CITED**


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**TABLE 1. Relative T7 burst sizes in temperature-sensitive gyrase mutants**

| Bacterial strain | Relevant genotype | Avg burst size* after preincubation at 43°C for: | | 10 min | 60 min |
|------------------|------------------|-----------------------------------------------|-------|--------|
| EC6 | Wild type | 1.48 (3) | 1.39 (3) |
| EC1512 | gyrB402(Ts) | 0.36 (2) | 0.06 (2) |
| EC1510 | gyrB41(Ts) | 0.81 (3) | 0.29 (3) |
| HF4704 | Wild type | 0.58 (2) | 0.55 (2) [0.41] |
| KNN453 | gyrA43(Ts) | 0.61 (2) | 0.24 (2) [0.25] |

* Values are average burst sizes normalized to burst sizes observed at 30°C; the number of experiments is shown in parentheses. Data shown in brackets are from Kreuzer and Cozzarelli (10).


