Involvement of Host DNA Gyrase in Growth of Bacteriophage T5

ANDREAS CONSTANTINOU,[1]† KAREN VOELKEL-MEIMAN,[2]‡ ROLF STERNGLANZ,[2] MAUREEN M. McCORQUODALE,[3] AND D. JAMES McCORQUODALE[1]*

Department of Biochemistry[1] and Departments of Pediatrics and Physiology,[3] Medical College of Ohio, Toledo, Ohio 43699, and Department of Biochemistry, State University of New York, Stony Brook, New York 11794[2]

Bacteriophage T5 did not grow at the nonpermissive temperature of 42°C in *Escherichia coli* carrying a temperature-sensitive mutation in *gyrB* [*gyrB*(Ts)], but it did grow in *gyrA*(Ts) mutants at 42°C. These findings indicate that the A subunit of host DNA gyrase is unnecessary, whereas the B subunit is necessary for growth of T5. The necessity for the B subunit was confirmed by a strong inhibition of T5 growth by novobiocin and coumermycin A_1, which interfere specifically with the function of the B subunit of host DNA gyrase. However, T5 growth was also strongly inhibited by nalidixic acid, which interferes specifically with the function of the A subunit. This inhibition was due to the interaction of nalidixic acid with the A subunit and not just to its binding to DNA, because appropriate mutations in the *gyrA* gene of the host conferred nalidixic acid resistance to the host and resistance to T5 growth in such a host. The inhibition by nalidixic acid was also not due to a cell poison formed between nalidixic acid and the A subunit (K. N. Kreuzer and N. R. Cozzarelli, J. Bacteriol. 140:424–435, 1979) because nalidixic acid inhibited growth of T5 in a *gyrA*(Ts) mutant (KNK453) at 42°C. We suggest that T5 grows in KNK453 at 42°C because its *gyrA*(Ts) mutation is leaky for T5. Inhibition of T5 growth due to inactivation of host DNA gyrase was caused mainly by inhibition of T5 DNA replication. In addition, however, late T5 genes were barely expressed when host DNA gyrase was inactivated.

DNA gyrase of *Escherichia coli* is a tetrameric protein consisting of two A subunits and two B subunits. It catalyzes the ATP-dependent conversion of relaxed or positively supertwisted DNA into negatively supertwisted DNA and is essential for DNA replication in *E. coli* (10, 12, 16). It is additionally involved in the regulation of transcription of some genes, since some promoters function with different efficiencies when they are supertwisted (8, 16, 22).

The role that host DNA gyrase plays in the growth and development of bacteriophages varies from case to case. It is not essential for bacteriophage T4 since this phage directs the synthesis of its own topoisomerase (19, 25), which is assembled from the polypeptides coded by genes 39, 52, and 60. However, inhibition of host gyrase reduces T4 DNA synthesis to some extent (3, 16), and mutants defective in genes 39, 52, or 60 are totally dependent upon the host gyrase (20). Bacteriophage T7 requires the B subunit of host DNA gyrase, but its requirement for the A subunit is controversial (3, 7, 15, 16, 24). Bacteriophages φX174 and N4 have absolute requirements for host gyrase (9, 16), and growth of λ, φR, and T5 is strongly inhibited by nalidixic acid (3). Bacteriophages SP82, SP50, and φ29, which use *Bacillus subtilis* as a host, are all relatively unaffected by nalidixic acid (3). However, SPO1, another *B. subtilis* bacteriophage, is inhibited by nalidixic acid at high concentrations and by novobiocin at low concentrations. It requires the B subunit of host DNA gyrase but modifies or replaces the A subunit with one that is still sensitive to nalidixic acid (2). Thus, inhibition of phage growth by nalidixic acid does not necessarily mean a requirement for the host A subunit.

In this paper, we report the role that host DNA gyrase plays in the growth of bacteriophage T5. We show that replication of T5 DNA and normal expression of some T5 genes require the host B subunit and probably also the host A subunit. Since inhibitors that interact with either the A subunit (e.g., nalidixic acid) or the B subunit (e.g., novobiocin) strongly inhibit replication of T5 DNA and production of mature T5 particles, and whereas thermal inactivation of the A subunit is not so definitive but thermal inactivation of the B-subunit is, we offer an interpretation of these inhibitory effects that is compatible with the B subunit being necessary and the A subunit possibly being necessary for T5 growth and development. We favor the interpretation that both the A and B subunits are necessary.

MATERIALS AND METHODS

Bacterial and phage strains. The bacterial strains used in this study, together with their properties and sources, are listed in Table 1. Bacteriophage T5[+] was from the stock collection of D.J.M.

Burst sizes in temperature-sensitive strains. *E. coli* strains KNK453, N4178, N99, HF4704, LE234, and LE316 were grown at 30°C to 2×10^8 cells per ml in Luria-Bertani broth containing 1 mM $CaCl_2$, harvested by centrifugation, and suspended in T5 adsorption buffer (0.05 M NaCl, 0.01 M Tris hydrochloride [pH 7.5], 0.001 M $CaCl_2$) at 5×10^9 cells per ml. The concentrated cell suspension was divided into two equal volumes. One volume was incubated for 30 min at 30°C before infection with T5 at a ratio of 0.5 phage per bacterial cell, whereas the other volume was incubated for 30 min at 42°C before infection in the same manner. Phage were allowed to adsorb for 15 min, and the phage-bacterium complexes were diluted into fresh Luria-Bertani broth plus 1 mM $CaCl_2$ equilibrated to the desired temperatures of 30 and 42°C, respectively. Aeration was begun, and samples were taken throughout the growth cycle to determine PFU. Burst sizes were calculated by dividing the concentration of PFU (progeny phage) at the end of lysis by the concentration of PFU (infected cells) before lysis started.

---

* Correspoding author.

† Present address: Department of Molecular Biology, Northwestern University Medical School, Chicago, IL 60611.

‡ Present address: Department of Biology, Yale University, New Haven, CT 06511.

TABLE 1. Strains of *E. coli*

Strain	Relevant genotype	Source (reference)
F	F⁻ T5ˢ	McCorquodale (21)
K-12 HF4704	F⁻ *polA thyA uvrA phx gyrA⁺* (parent of KNK453)	Kreuzer and Cozzarelli (16)
K-12 KNK453	F⁻ *polA thyA uvrA phx gyrA43*(Ts)	Kreuzer and Cozzarelli (16)
K-12 N4156	F⁻ *polA end thy gyrA* Nalʳ	Sternglanz (5)
K-12 N99	F⁻ *galK2 recB rpsL gyrB⁺* Bgl⁻ (parent of N4178)	National Institutes of Health
K-12 N4178	F⁻ *galK2 recB rpsL gyrB203*(Ts) *gyrB221* Couʳ Bgl⁺	Gellert (11)
K-12 LE234	F⁻ *ilv argH metB rpsL tna thi xyl gyrB⁺* (parent of LE316)	E. Orr
K-12 LE316	F⁻ *ilv argH metB rpsL tna thi xyl gyrB*(Ts) (selected as chlorobiocin resistant)	E. Orr

When nalidixic acid was present during determinations of average burst size in these temperature-sensitive strains, it was added at zero time (final concentration, 10 μg/ml), which was defined as the time at which the phage-bacterium complexes were diluted into phage growth medium. Strain N4156 (Nalʳ) was used in the same manner as were the temperature-sensitive strains except that burst sizes were determined at 37°C.

Rates of DNA synthesis in temperature-sensitive strains. Cells were grown, harvested, infected, and diluted for phage growth as described above except that the growth medium for cells and phage was M-9 supplemented with 0.5 mM CaCl₂ 10 μg each of 18 amino acids (4) per ml, and 40 μg of freshly prepared cysteine per ml; suspension was in M-9 adsorption buffer (4), and the input ratio was 10 to 15 phage per bacterial cell. Samples of bacteria were taken before the addition of phage to determine the concentration of input bacteria. The concentrations of uninfected cells and free phage were determined at 10 min after infection. The concentration of uninfected cells was 5% or slightly less when K-12 strains were used. Rates of DNA synthesis in the infected cultures at 30 and 42°C were determined by measuring the amount of [*methyl*-³H]thymidine incorporated into DNA during a 2-min labeling period. Samples (1 ml) were removed from each infected culture at specified times after infection and added to tubes equilibrated at 30 and 42°C, respectively, containing 3.3 μCi of [*methyl*-³H]thymidine (60 Ci/mol), 20 μg of 2'-deoxyadenosine, and 4 μg of uracil all in a total volume of 0.2 ml of growth medium. After a 2-min labeling period with aeration and at the respective temperature, 1 ml of ice-cold 10% trichloroacetic acid (TCA) was mixed into the contents of the tube. The samples were chilled for 10 min, and the precipitates were collected on nitrocellulose filters (no. BA85; diameter, 25 mm; pore size, 0.45 μm; Schleicher & Schuell, Inc.). The precipitates on the filters were washed four times with 3 ml of 5% TCA and once with 1% TCA. After drying, the radioactivity on the filters was counted in Scinti-Verse universal cocktail in a liquid scintillation counter.

Dose-response curves. The response of T5-infected cells to various concentrations of inhibitors was measured by average burst sizes, which were determined from one-step growth curves. *E. coli* F was grown to 2 × 10⁸ cells per ml in nutrient broth (1), harvested by centrifugation, and suspended in T5 adsorption buffer at 5 × 10⁹ cells per ml. The adsorption buffer contained, when desired, nalidixic acid, novobiocin, or other inhibitors. Stock solutions of novobiocin were prepared in sterile distilled H₂O, whereas stock solutions of nalidixic acid were prepared in 0.01 N NaOH. Control cultures were treated with the same volume of H₂O or 0.01 N NaOH that was used to add the inhibitors to experimental cultures. The concentrated cells were infected with T5⁺ at a ratio of five phage per bacterial cell. The phage were allowed to adsorb for 5 min at 37°C, the phage-bacterium complexes were diluted 25-fold (to 2 × 10⁸ infected cells per ml) into nutrient broth at 37°C containing 1 mM CaCl₂ and, when desired, inhibitors at predetermined concentrations, and aeration was begun. The concentration of uninfected cells was always less than 1% under these conditions. Burst sizes were calculated as described for the temperature-sensitive strains.

Rates of DNA and RNA synthesis in the presence of inhibitors. *E. coli* F was grown at 37°C to 2.0 × 10⁸ cells per ml in M-9 medium supplemented with 10 μg each of 18 amino acids (4) per ml and 0.5 mM CaCl₂, harvested by centrifugation, and suspended at 0°C in M-9 adsorption buffer at 4 × 10⁹ cells per ml. The M-9 adsorption buffer contained nalidixic acid or novobiocin at the desired concentrations. The concentrated cells were infected with T5⁺ at a ratio of five phage per bacterial cells. Phage were allowed to adsorb for 5 min at 37°C; the phage-bacterium complexes were diluted 10-fold (to 4 × 10⁸ cells per ml) into M-9 medium at 37°C supplemented with 18 amino acids, 0.5 mM CaCl₂, and nalidixic acid or novobiocin at the desired concentration; and aeration was begun. Rates of DNA synthesis were estimated by measuring the amount of [*methyl*-³H]thymidine incorporated into DNA during a 2-min labeling period as described for the temperature-sensitive strains.

Rates of RNA synthesis were similarly estimated by measuring the amount of [5-³H]uridine incorporated into RNA during a 2-min labeling period. Samples (0.8 ml) were removed from the culture of infected bacteria at specified times after infection and added to tubes containing 3.3 μCi of [5-³H]uridine (28 Ci/mmol) and nalidixic acid or novobiocin at the same concentration as in the original culture in a volume of 0.2 ml of growth medium. The 2-min labeling period was at 37°C and was terminated by the addition of 1 ml of ice-cold 10% TCA. The precipitated RNA was collected, washed on nitrocellulose filters, and counted as described for DNA.

Cumulative incorporation of ¹⁴C-amino acids into protein. *E. coli* F was grown, harvested, suspended, and infected as described for experiments to determine rates of DNA and RNA synthesis. After infection, the phage-bacterium complexes were diluted to 2 × 10⁸/ml into M-9 medium at 37°C supplemented with 5 μg each of 18 amino acids per ml, 0.5 mM CaCl₂, and 0.3 μCi of a mixture of L-U-¹⁴C-amino acids (specific activity, 1.78 mCi/mg) per ml. At specified times after this dilution, which was designated zero time for the infection, 1-ml volumes were removed and added to 4.5 ml of 6.25% TCA. The resulting suspensions were heated at 90°C for 15 min, and the precipitates were collected and washed on nitrocellulose filters (no. BA85; diameter, 25 mm; pore size, 0.45 μm; Schleicher & Schuell, Inc.). Four washes with 5% TCA and one with 1% TCA were used. Counting was done as described for labeled DNA and RNA.

TABLE 2. Average burst sizes of bacteriophages in *gyrA* and *gyrB* hosts

Strain	Infecting phage	Burst sizes [(PFU concn after lysis)/(PFU concn before lysis)] for growth conditions					
		30°C		42°C		37°C	
		Control	Nalidixic acid added	Control	Nalidixic acid added	Control	Nalidixic acid added
HF4704 (*gyrA*⁺)	T5	596	<1	468	6	ND[a]	ND
	ΦX174	532	ND	405	ND	ND	ND
KNK453 [*gyrA*(Ts)]	T5	636	<1	304	<1	ND	ND
	ΦX174	678	ND	<1	ND	ND	ND
N99 (*gyrB*⁺)	T5	200	ND	93	ND	ND	ND
N4178 [*gyrB*(Ts)]	T5	215	ND	<1	ND	ND	ND
LE234 (*gyrB*⁺)	T5	143	ND	161	ND	ND	ND
LE316 [*gyrB*(Ts)]	T5	235	ND	<1	ND	ND	ND
N4156 (Nalʳ)	T5	ND	ND	ND	ND	220	114

[a] ND, Not done.

Preparation of radioactively labeled extracts for polyacrylamide gel electrophoresis. The temperature-sensitive strains KNK453 and N4178 were grown at 30°C in M-9 supplemented with 0.5 mM $CaCl_2$ and 17 amino acids (L-leucine was omitted) to 2×10^8 cells per ml. For experiments at 30°C, the concentrated cells were infected at 30°C with T5 at a ratio of 15 phage per bacterial cell. For experiments at 42°C, the concentrated cells were incubated for 60 min at 42°C and then infected at 42°C with T5 at the same phage-to-bacterium ratio as for the culture at 30°C. For both experiments, the phage were allowed to adsorb for 10 min, and the phage-bacterium complexes were diluted into 24 volumes of M-9 medium that was supplemented with 0.5 mM $CaCl_2$ and 17 amino acids; the medium had been previously adjusted to the temperature used in the experiment. Aeration was begun at the time of this dilution (zero time).

At specific times after infection, a 5-ml sample was withdrawn from the liquid culture, quickly mixed with 0.2 ml of a solution containing 1 μCi of L-[U-^{14}C]leucine (specific activity, 270 to 300 mCi/mmol) in a test tube, and aerated for 5 min at the temperature of the experiment. At the end of each labeling period, the radioactive samples were cooled quickly over ice to 0°C and centrifuged at $12,000 \times g$ for 15 min at 0 to 4°C. The pellets were suspended in 0.5 ml of sample preparation buffer (0.05 M Tris hydrochloride [pH 6.8], 1% sodium dodecyl sulfate [SDS], 1% 2-mercaptoethanol, 10% glycerol, 0.003% bromophenol blue), and solubilized by heating for 2 min at 100°C.

Polyacrylamide gel electrophoresis was performed by the method of Laemmli (18). Stacking gels contained 5% acrylamide, 0.064% bisacrylamide, 0.1% SDS, and 0.63 M Tris hydrochloride (pH 6.8). Running gels contained 15% acrylamide, 0.4% bisacrylamide, 0.1% SDS, and 0.63 M Tris hydrochloride (pH 6.8). Polymerization of acrylamide solutions was catalyzed by 0.05% N,N,N',N'-tetramethylethylenediamine plus 0.1% ammonium persulfate for the stacking gels and 0.03% N,N,N',N'-tetramethylethylenediamine plus 0.03% ammonium persulfate for the running gels. Electrode buffer contained 0.050 M Tris hydrochloride, 0.383 M glycine, and 0.1% SDS, at pH 8.3. Electrophoresis was performed at 4°C with a constant current of 17 mA for 4 to 5 h. After electrophoresis, the gels were fixed in 10% TCA for 30 min, stained in Coomassie brilliant blue R250

(no. 14013; 0.123% Coomassie brilliant blue in 7.5% glacial acetic acid–50% methanol; Eastman Chemical Products, Inc.) for 2 h, destained in 7.5% acetic acid, and dried on Whatman 3MM filter paper for autoradiography. Autoradiography was performed with Kodak DF-85 dental X-ray film.

RESULTS

When T5⁺ infected a *gyrB*(Ts) mutant, a productive infection ensued at 30°C but not at 42°C (Table 2). When T5⁺ infected a *gyrA*(Ts) mutant, however, a productive infection ensued at both the permissive (30°C) and the nonpermissive (42°C) temperatures (Table 2). To confirm that the *gyrA*(Ts) mutant used (KNK453) was indeed temperature sensitive, we showed that ΦX174 would not grow in it at 42°C (Table 2). These combined data suggest that the A subunit is unnecessary but the B subunit is necessary for a productive T5 infection.

The apparently nonessential role that the A subunit plays in a T5 infection was further illustrated by the abundant synthesis of T5 DNA when the A subunit was thermally denatured in a *gyrA*(Ts) mutant (Fig. 1). In contrast, T5 DNA synthesis in a *gyrB*(Ts) mutant was severely inhibited at 42°C (Fig. 2).

In contrast to the experiments with temperature-sensitive mutants, experiments with inhibitors of DNA gyrase indicated that both the A and B subunits of host DNA gyrase were necessary for T5 growth. Burst sizes in the presence of nalidixic acid, an inhibitor that interacts with the A subunit, were 50% of normal at 0.3 μg/ml and only 1% of normal at 1 μg/ml (Fig. 3). Similar results were obtained with oxolinic acid (data not shown). These findings imply that the A subunit is necessary for a productive T5 infection. Burst sizes in the presence of novobiocin, an inhibitor that interacts with the B subunit, were 50% of normal at 0.3 μg/ml and only 1% of normal at 1 μg/ml (Fig. 3). Coumermycin A_1 gave similar results (data not shown). These findings are in agreement with the results from the *gyrB*(Ts) mutants that indicate the B subunit is necessary for a productive T5 infection but not with the results from the *gyrA*(Ts) mutants. The inhibition of T5 growth with nalidixic acid that we observed was due to an interference with host DNA gyrase
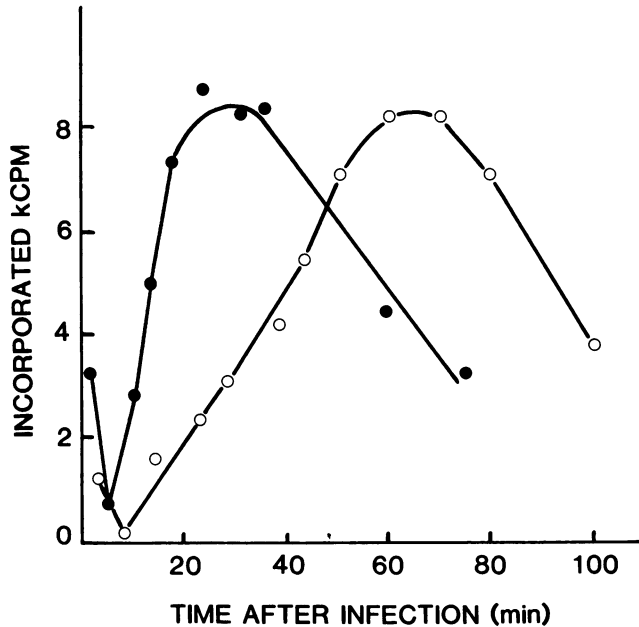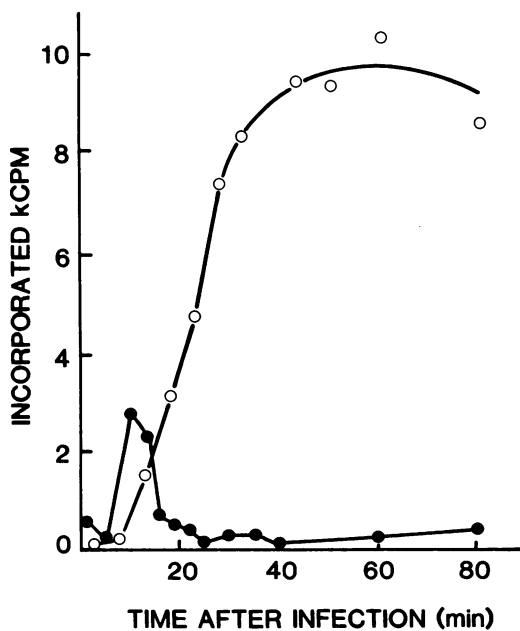
FIG. 1. Incorporation of [^{3}H]thymidine into DNA by strain KNK453 [gyrA(Ts)] after infection with bacteriophage T5 at 30°C (O) and at 42°C (●).

and not due just to the direct binding of nalidixic acid to T5 DNA (23), because nalidixic acid-resistant host mutants that contained a mutation in the gyrA gene allowed growth of T5 in the presence of nalidixic acid (Table 2).

Kreuzer and Cozzarelli (16) have reported that nalidixic acid does not inhibit production of T7 in hosts containing a thermolabile A subunit at a nonpermissive temperature (42°C) but does inhibit at a permissive temperature (30°C). In

the case of T5, nalidixic acid inhibited T5 production at both permissive and nonpermissive temperatures (Table 2).

The presence of either nalidixic acid or novobiocin reduced the synthesis of DNA, RNA, and protein in T5-infected cells but inhibited DNA synthesis most strongly. DNA synthesis was virtually abolished in the presence of 10 μg of nalidixic acid per ml (Fig. 4), as was the burst (Table 2). Nalidixic acid at a concentration of two μg/ml severely inhibited DNA synthesis and greatly lowered the burst size. Similarly, 1 μg of novobiocin per ml severely inhibited DNA synthesis and the burst size, and 10 to 20 μg of novobiocin per ml virtually abolished DNA synthesis (Fig. 5) and a measurable burst. Hence, the main effect of these inhibitors was to interfere with DNA synthesis; novobiocin did so by interacting with the B subunit of host DNA gyrase, and nalidixic acid did so by a mechanism that is open to interpretation and will be discussed later.

Synthesis of T5 DNA started about 9 min after infection at 37°C, and the amount of DNA synthesized reflected the time at which nalidixic acid or novobiocin was added. For example, when nalidixic acid was added at any time before T5 DNA synthesis started, the burst size was only about 3 to 5% of normal. When it was added after T5 DNA synthesis commenced, the burst size increased as more time for DNA synthesis was allowed before the inhibitor was added (Fig. 6).

RNA and protein synthesis were reduced in the presence of either novobiocin or nalidixic acid, but to a much lesser degree than was DNA synthesis. In the presence of 20 μg of novobiocin per ml, RNA synthesis was inhibited only about 50% at early times and about 30% at late times (Fig. 7). Severe inhibition of RNA synthesis was attained only at novobiocin concentrations of 100 μg/ml or more. Similar results were obtained with nalidixic acid.

Protein synthesis was also inhibited to a much lesser

FIG. 2. Incorporation of [^{3}H]thymidine into DNA by strain N4178 [gyrB(Ts)] after infection with bacteriophage T5 at 30°C (O) and at 42°C (●).
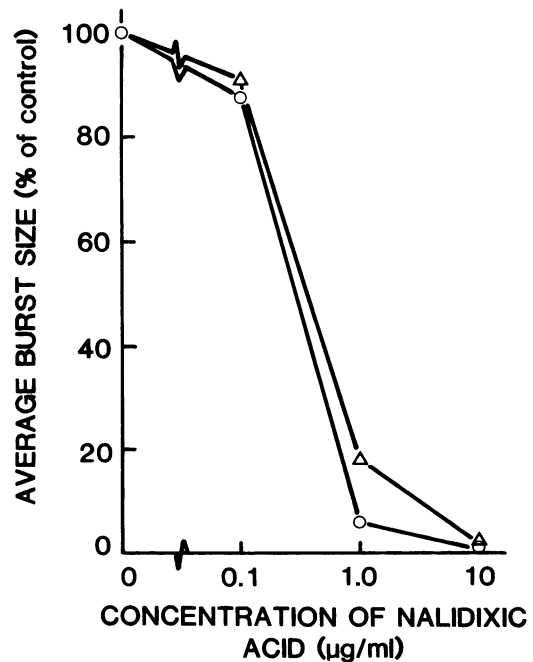

FIG. 3. Dose-response curves for development of bacteriophage T5 in E. coli F in the presence of nalidixic acid (O) or novobiocin (△). The inhibitors were added at the indicated concentrations 5 min before infection.

degree than was DNA synthesis in the presence of either novobiocin or nalidixic acid (Fig. 8). In the presence of 10 μg of nalidixic acid per ml, protein synthesis was inhibited only about 40% from 10 to 60 min after infection, and 100 μg of nalidixic acid per ml was required to inhibit the rate of protein synthesis by about 75%. During the first 5 to 7 min after infection, protein synthesis was inhibited even less than at later times, since 10 μg of nalidixic acid per ml caused only 18% inhibition and 100 μg/ml caused only 54% inhibition. We view this inhibition of protein synthesis as an indirect effect of the action of these inhibitors on the process of transcription.

Measurement of the degree of inhibition of total RNA and protein synthesis did not reveal the degree of inhibition of individual RNA and protein species. As already mentioned, the activity of some promoters may be sensitive to the degree of torsional stress that they possess. We, therefore, examined the synthesis of individual proteins by the method of pulse-labeling followed by autoradiography of the labeled proteins after separation in SDS gels. Some late proteins were not synthesized in a gyrB(Ts) mutant at 42°C but were synthesized in a gyrA(Ts) mutant at 42°C (Fig. 9).

The autoradiographic analyses of T5 protein synthesis also demonstrated that host DNA gyrase was not involved in transfer of T5 DNA into host cells since early proteins were synthesized when either the A or B subunit was thermally inactivated before infection.
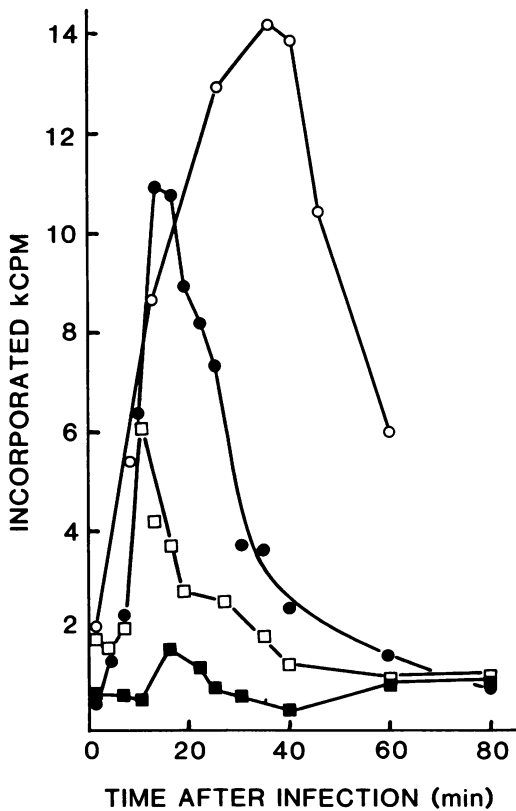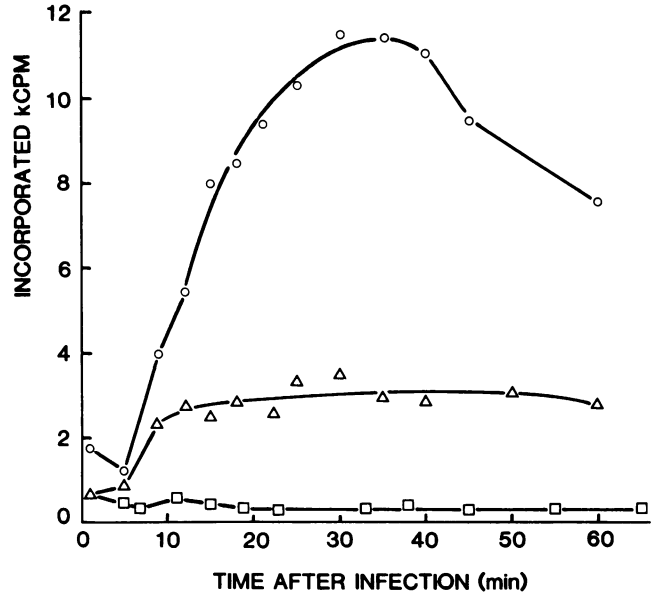

FIG. 5. Rates of incorporation of [³H]thymidine into DNA in *E. coli* F at increasing times after infection with bacteriophage T5 in the absence (O) or presence of novobiocin at 1 μg/ml (△) or 20 μg/ml (□). Each point represents the amount of [³H]thymidine incorporated into DNA during a 2-min incubation starting at the time after infection indicated along the abscissa.

DISCUSSION

The results presented in this paper show that growth of bacteriophage T5 in *E. coli* required the B subunit and possibly also the A subunit of host DNA gyrase. The requirement for the B subunit was demonstrated by using gyrB(Ts) mutants, which did not support growth of T5 at the nonpermissive temperature of 42°C, and by using inhibitors

FIG. 4. Rates of incorporation of [³H]thymidine into DNA in *E. coli* F at increasing times after infection with bacteriophage T5 in the absence (O) or presence of nalidixic acid at 1 μg/ml (●), 2 μg/ml (□), or 10 μg/ml (■). Each point represents the amount of [³H]thymidine incorporated into DNA during a 2-min incubation starting at the times after infection indicated along the abscissa.
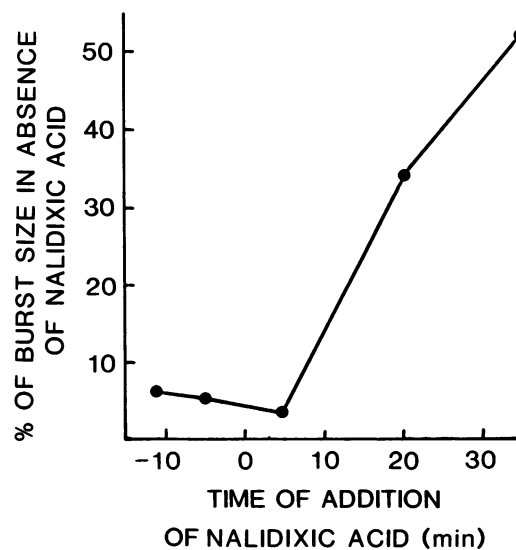

FIG. 6. Effect of time of addition of nalidixic acid on burst size of bacteriophage T5 in *E. coli* F. Nalidixic acid was added 11 or 5 min before infection (− values) or 5, 20, or 35 min after infection (+ values).
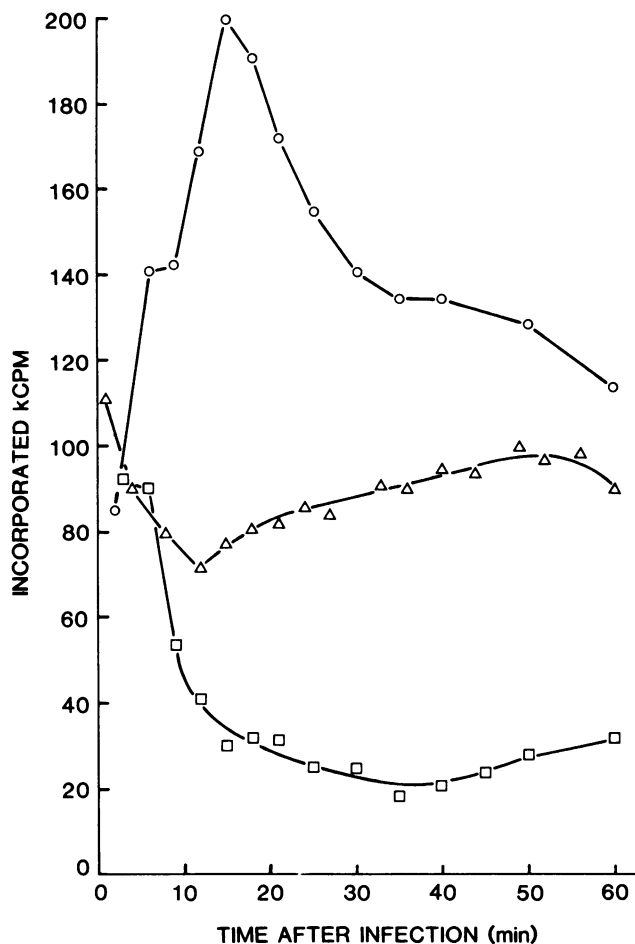
FIG. 7. Rates of incorporation of [5-^{3}H]uridine into RNA in *E. coli* F at increasing times after infection with bacteriophage T5 in the absence (O) or presence of novobiocin at 20 μg/ml (△) or 100 μg/ml (□). Each point represents the amount of [5-^{3}H]uridine incorporated into RNA during a 2-min incubation starting at the time after infection indicated along the abscissa.

is to assume that the A subunit of host DNA gyrase is not necessary for growth of T5 and that the inhibition of T5 growth by nalidixic acid is due to the cell poison proposed by Kreuzer and Cozzarelli (16). These authors proposed that a complex, which forms between the A subunit and nalidixic acid, is held tightly to DNA, causes lesions in the DNA, and thereby renders the DNA incapable of functioning normally. Thus, inhibition of phage growth by nalidixic acid results even though the A subunit is unnecessary. In the case of a thermolabile A subunit from a *gyrA*(Ts) host, no inhibition would be observed at 42°C because nalidixic acid cannot interact with a thermally denatured A subunit to form such a cell poison. However, since growth of T5 was inhibited by nalidixic acid at 42°C, when the *gyrA43* subunit was thermally inactivated (Table 2), this model does not explain the present results. Indeed, the results presented here indicate that, if the cell poison proposed by Kreuzer and Cozzarelli exists, it appears not to act on all systems. Furthermore, this second model requires a substitute for the A subunit of the host because the B subunit by itself has no known function (14). A possible candidate would be a T5-coded subunit; however, such a subunit would have to be normally sensitive to nalidixic acid but become insensitive when mutations in the host *gyrA* gene that confer resistance to nalidixic acid on the host are present. This seems an unlikely circumstance.

An alternative explanation for the sensitivity of T5 growth to nalidixic acid in *gyrA*(Ts) hosts at 42°C is that T5 induces a modification of the host A subunit such that it is no longer temperature sensitive. Such an explanation would require the thermally inactivated A subunit to be reactivated by this putative modification, because our experimental procedure was to infect after the A subunit had been thermally inactivated for as long as 1 h. Although one could consider that a modification of a native protein might increase its thermal stability, it seems less likely that a thermally denatured protein could act as a substrate for such a modification and
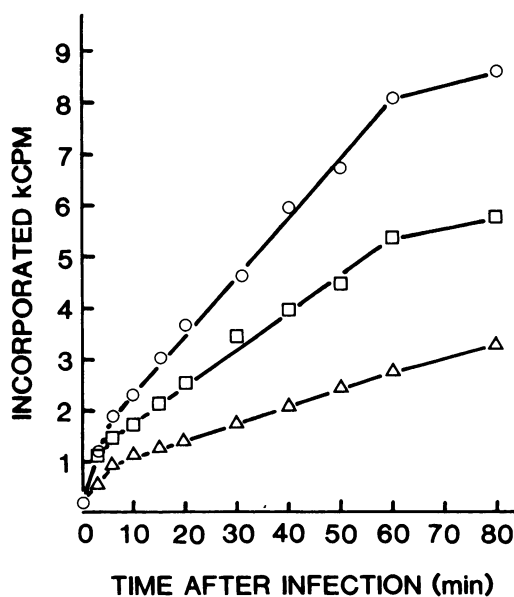
such as novobiocin and coumermycin A$_1$, which blocked the growth of T5 by interacting specifically with and inactivating the B subunit.

T5 did grow in *gyrA*(Ts) mutants at the nonpermissive temperature of 42°C, a finding that appears to demonstrate the nonessential nature of the A subunit of host DNA gyrase for T5 growth, although the A subunit is essential for growth of the uninfected host cells. However, inhibitors such as nalidixic acid and oxolinic acid, which interact specifically with the A subunit to inhibit its normal function, blocked the growth of T5.

These apparently contradictory results can be reconciled in at least two ways. First, we may assume that the *gyrA*(Ts) strain [KNK453 (*gyrA43*)] is leaky (24) and that, although thermal denaturation of the A subunit of its DNA gyrase at 42°C did not leave sufficient functional DNA gyrase for growth of the uninfected host or of bacteriophage φX174, it did leave enough for growth of T5. If such is the case, addition of nalidixic acid at the nonpermissive temperature of 42°C should eliminate the growth of T5, which is what we observed.

A second model that might appear to reconcile the results

FIG. 8. Cumulative incorporation of ^{14}C-amino acids into protein in *E. coli* F after infection with bacteriophage T5 in the absence (O) or presence of nalidixic acid at 10 μg/ml (□) or 100 μg/ml (△). The inhibitor, when present, was added 5 min before infection.
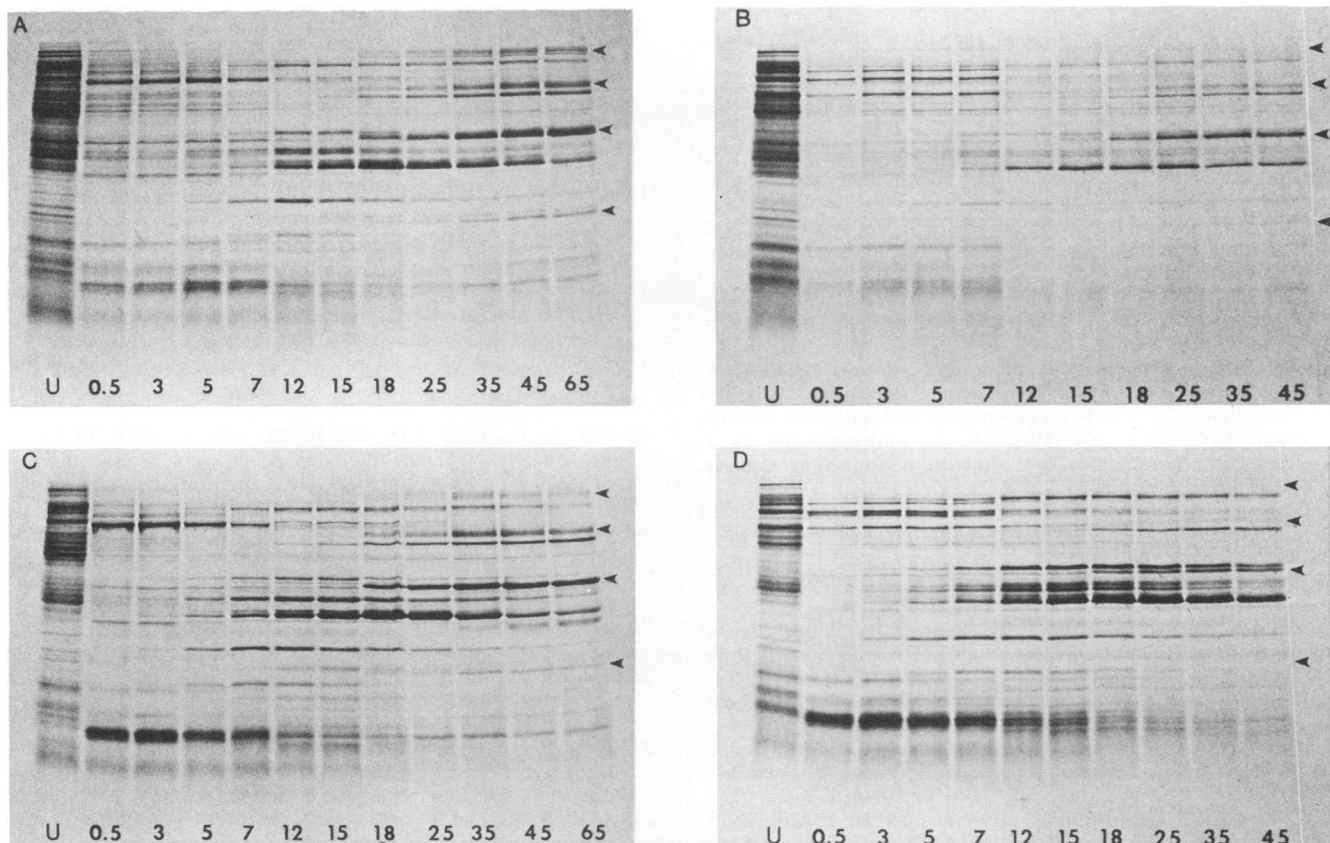
FIG. 9. Autoradiograms of electrophoretically separated ^{14}C-labeled polypeptides synthesized in KNK453 [gyrA(Ts)] at 30°C (A), KNK453 [gyrA(Ts)] at 42°C (B), N4178 [gyrB(Ts)] at 30°C (C), and N4178 [gyrB(Ts)] at 42°C (D) after infection with bacteriophage T5. The number below each lane specifies the time after infection at which a 5-min labeling period at the indicated temperature was begun. The autoradiographic patterns of ^{14}C-labeled polypeptides synthesized in uninfected cells are designated U. The arrowheads to the right of the rightmost lanes designate the positions of polypeptides (mostly late) that are most affected by the absence of a functional B subunit of host DNA gyrase.

then become renatured. However, we do not discard this possibility entirely.

We favor the first model because it requires fewer assumptions and is in general agreement with the report (24) that bacteriophage T7 can also grow at 42°C in the same gyrA(Ts) host [KNK453 (gyrA43)] that we have used. The authors of this report also suggest that KNK453 (gyrA43) is leaky at 42°C. If the first model is correct, then we suggest that temperature-sensitive experiments with KNK453 (gyrA43) be interpreted with the knowledge that T5 and T7 will grow in it at 42°C, whereas φX174 and the strain itself will not. Different bacteriophages appear to require different amounts of host DNA gyrase, with N4, φX174, and probably λ requiring high levels, T5 and T7 being able to grow with lower levels, and T4 being able to generate a moderate burst without any host DNA gyrase. Obviously, the growth of other phages in the temperature-sensitive host, KNK453 (gyrA43), at 42°C will have to be examined on an individual basis.

A major effect of interference with the function of DNA gyrase in T5-infected cells is the inhibition of T5 DNA synthesis. However, we have also observed that expression of some late genes is strongly affected when DNA gyrase activity is inhibited. The inhibition of late gene expression by inhibitors of DNA replication is apparently not a consequence of a coupling between late transcription and DNA

replication, because late genes are expressed in the absence of T5 DNA replication (13). The involvement of DNA gyrase with expression of late T5 genes may be due to a requirement for torsional stress for transcriptional initiation of these genes. Changes in the degree of DNA supercoiling are known to affect the function of many E. coli promoters (for a recent review, see reference 8). Torsional stress due to underwinding might have a greater effect on the rate of transcriptional initiation from promoters that are GC rich compared with those that are AT rich. The finding that the base composition of a late T5 promoter is relatively GC rich (56% GC) (17), whereas early T5 promoters, which function well in the presence of nalidixic acid, are AT rich (75 to 80% AT) (6), would be consistent with this interpretation. This requirement for torsional stress in the portion of T5 DNA used for late T5 transcription seems as important for efficient expression of late T5 genes as is the requirement for the noncovalent modification of the host RNA polymerase by the product of early gene C2, presumably to use late T5 promoters efficiently (21).

LITERATURE CITED

1. Adams, M. H. 1959. Bacteriophages. Interscience Publishers, Inc., New York.
2. Alonso, J. C., A. N. Sarachu, and O. Grau. 1981. DNA gyrase inhibitors block development of *Bacillus subtilis* bacteriophage SPO1. J. Virol. 39:855–860.
3. Baird, J. P., G. J. Bourguignon, and R. Sternglanz. 1972. Effect of nalidixic on the growth of deoxyribonucleic acid bacteriophages. J. Virol. 9:17–21.
4. Beckman, L. D., M. S. Hoffman, and D. J. McCorquodale. 1971. Pre-early proteins of bacteriophage T5: structure and function. J. Mol. Biol. 62:551–564.
5. Bourguignon, G. J., M. Levitt, and R. Sternglanz. 1973. Studies on the mechanism of action of nalidixic acid. Antimicrob. Agents Chemother. 4:479–486.
6. Bujard, H., A. Niemann, K. Breunig, U. Roisch, A. Dresel, A. von Gabain, R. Gentz, D. Stueber, and H. Weiher. 1982. The interaction of *E. coli* RNA polymerase with promoters of high signal strength, p. 121–140. *In* R. L. Rodriguez and M. J. Chamberlin (ed.), Promoters: structure and function. Praeger, New York.
7. deWyngaert, M. A., and D. C. Hinkle. 1979. Involvement of DNA gyrase in replication and transcription of bacteriophage T7 DNA. J. Virol. 29:529–535.
8. Drlica, K. 1984. Biology of bacterial deoxyribonucleic acid topoisomerases. Microbiol. Rev. 48:273–289.
9. Falco, S. C., R. Zivin, and L. B. Rothman-Denes. 1978. Novel template requirements of N4 virion RNA polymerase. Proc. Natl. Acad. Sci. USA 75:3220–3224.
10. Gellert, M. 1981. DNA topoisomerases. Annu. Rev. Biochem. 50:897–910.
11. Gellert, M., L. M. Fisher, and M. H. O'Dea. 1979. DNA gyrase: purification and catalytic properties of a fragment of gyrase B protein. Proc. Natl. Acad. Sci. USA 76:6289–6293.
12. Gellert, M., K. Mizuuchi, M. H. O'Dea, and H. A. Nash. 1975. DNA gyrase: an enzyme that introduces superhelical turns into DNA. Proc. Natl. Acad. Sci. USA 73:3872–3876.
13. Hendrickson, H. E., and D. J. McCorquodale. 1971. Genetic and physiological studies of bacteriophage T5. 2. The relationship between phage DNA synthesis and protein synthesis in T5-infected cells. Biochem. Biophys. Res. Commun. 43:735–740.
14. Higgins, N. P., C. L. Peebles, A. Sugino, and N. R. Cozzarelli. 1978. Purification of subunits of *Escherichia coli* DNA gyrase and reconstitution of enzymatic activity. Proc. Natl. Acad. Sci. USA 75:1773–1777.
15. Itoh, T., and J. I. Tomizawa. 1977. Involvement of DNA gyrase in bacteriophage T7 DNA replication. Nature (London) 270:78–80.
16. 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.
17. Ksenzenko, V. N., T. P. Kamynina, N. M. Pustoshilova, V. M. Kryukov, and A. A. Bayev. 1982. Cloning of bacteriophage T5 promoters. Mol. Gen. Genet. 185:520–522.
18. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) 227:680–685.
19. Liu, L. F., C.-C. Liu, and B. M. Alberts. 1979. T4 DNA topoisomerase: a new ATP-dependent enzyme essential for initiation of T4 bacteriophage DNA replication. Nature (London) 281:456–461.
20. McCarthy, D. 1979. Gyrase-dependent initiation of bacteriophage T4 DNA replication: interactions of *Escherichia coli* gyrase with novobiocin, coumermycin and phage DNA-delay gene products. J. Mol. Biol. 127:265–283.
21. McCorquodale, D. J. 1975. The T-odd bacteriophages. Crit. Rev. Microbiol. 4:101–159.
22. Menzel, R., and M. Gellert. 1983. Regulation of the genes for *E. coli* DNA gyrase; homeostatic control of DNA supercoiling. Cell 34:105–113.
23. Shen, L. L., and A. G. Pernet. 1985. Mechanism of inhibition of DNA gyrase by analogues of nalidixic acid: the target of the drugs is DNA. Proc. Natl. Acad. Sci. USA 82:307–311.
24. Steck, T. R., and K. Drlica. 1985. Involvement of DNA gyrase in bacteriophage T7 growth. J. Virol. 53:296–298.
25. Stetler, G. L., G. J. King, and W. M. Huang. 1979. T4 DNA-delay proteins, required for specific DNA replication, form a complex that has ATP-dependent DNA topoisomerase activity. Proc. Natl. Acad. Sci. USA 76:3737–3741.