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# Involvement of DNA Gyrase in Replication and Transcription of Bacteriophage T7 DNA

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Growth of bacteriophage T7 is inhibited by the antibiotic coumermycin $A_1$, an inhibitor of the *Escherichia coli* DNA gyrase. Since growth of the phage is insensitive to the antibiotic in strains containing a coumermycin-resistant DNA gyrase, this enzyme appears to be required for phage growth. We have investigated the effect of coumermycin on the kinetics of DNA, RNA, and protein synthesis during T7 infection. DNA synthesis is completely inhibited by the antibiotic. In addition, coumermycin significantly inhibits transcription of late but not early genes. Thus, *E. coli* DNA gyrase may play an important role in transcription as well as in replication of T7 DNA.

The *Escherichia coli* enzyme DNA gyrase introduces negative superhelical turns into a circular, double-stranded DNA in an ATP-dependent reaction. The genetic locus (*cou*) which determines resistance to coumermycin $A_1$ and to novobiocin has been identified as controlling the activity of DNA gyrase (5). DNA gyrase isolated from wild-type cells is inhibited by both these antibiotics, while enzyme from a $Cou^r$ strain is resistant to inhibition. More recently, a second subunit of DNA gyrase has been identified as the product of *nalA*, a different genetic locus which determines resistance to nalidixic acid and to oxolinic acid (22). Thus, DNA gyrase appears to be the specific target for inhibition by all four of these antibiotics.

It has been known for some time that nalidixic acid inhibits the replication of bacteriophage T7 DNA (1). The recent identification of the *nalA* protein as a subunit of DNA gyrase suggests that this novel enzyme plays an essential role in T7 DNA replication. Consistent with this, Itoh and Tomizawa have recently shown that T7 DNA replication is also sensitive to the antibiotic coumermycin (8).

We have investigated the effect of coumermycin on bacteriophage T7 nucleic acid synthesis. Our results indicate that *E. coli* DNA gyrase is required for efficient transcription of many of the late phage genes, as well as for phage DNA synthesis.

## MATERIALS AND METHODS

**Bacterial strains and bacteriophage.** The *E. coli* K-12 strains NI 748 (coumermycin resistant) and NI 747 (coumermycin sensitive), which are parallel transductants of CRT46 (5), were obtained from M. Gellert. *E. coli* B/1 was from M. Chamberlin. T7 wild-type and amber mutants were obtained from F. W. Studier. For convenience, T7 mutants are designated by gene only. The amber mutations used are gene 1, *am* 193; gene 5, *am* 28; gene 6, *am* 147.

**Chemicals.** Coumermycin $A_1$ (referred to as coumermycin), lot 448, was a gift of W. F. Minor, Bristol Laboratories, Syracuse, N.Y. Coumermycin was added to cultures from a stock solution (10 mg/ml) in dimethyl sulfoxide. [$^3$H]thymidine (20 Ci/mmol) and [$^3$H]uridine (20 Ci/mmol) were from New England Nuclear Corp., and $^{14}$C-labeled L-amino acid mixture (50 mCi/mAtom of carbon) was from Amersham.

**Kinetics of DNA, RNA, and protein synthesis.** Bacteria were grown in a gyratory shaker at 30°C in M9 medium or, for the K-12 strains NI 747 and NI 748, in M9 medium supplemented, per ml, with 2 μg of thymine, 5 μg of thiamine, and 60 μg each of threonine, valine, leucine, and isoleucine. At a density of $2.5 \times 10^8$ cells per ml, bacteria were infected with T7 phage at a multiplicity of 10. To measure the rate of DNA, RNA, or protein synthesis, 0.1-ml samples were removed from the culture and placed in tubes containing 10 μl of [$^3$H]thymidine (50 μCi/ml), [$^3$H]uridine (50 μCi/ml), or $^{14}$C-labeled amino acids (5 μCi/ml). After 1 min at 30°C, synthesis was terminated by the addition of 3 ml of cold 10% trichloroacetic acid. The precipitates were collected on Whatman GF/C filters and washed five times with 3 ml of cold 1 N HCl and finally with 3 ml of cold ethanol. Radioactivity on the dried filters was determined in a toluene-based solvent in a liquid scintillation counter.

**UV irradiation.** For experiments in which bacteria were irradiated with UV light prior to T7 infection, 10 ml of bacteria ($2.5 \times 10^8$ cells per ml) was placed in a sterile open petri dish (12 by 100 mm) and irradiated at 254 nm with a General Electric G8T5 germicidal lamp for 2.5 min at a fluence of 3.3 J/m$^2$ per s. The cells were then returned to a sterile flask and incubated in a gyratory shaker at 30°C for 20 min prior to infection.

## RESULTS

**Coumermycin inhibits growth of bacteriophage T7.** The effect of coumermycin on the growth of phage T7 in *E. coli* B/1 and in the isogenic K-12 strains NI 747 Cou$^s$ and NI 748 Cou$^r$ is shown in Fig. 1. In 10-μg/ml coumermycin, the burst size in B/1 was reduced to 6% of the control. Growth in both K-12 strains was somewhat less sensitive to the drug, but at 10 μg/ml the burst in the coumermycin-sensitive strain was reduced to 20% of the control, whereas growth in the resistant strain was unaffected. Since the coumermycin-resistant strain NI 748 has been shown to contain a coumermycin-resistant DNA gyrase (5), these results suggest that the *E. coli* DNA gyrase plays an essential role in bacteriophage growth.

**Effect of coumermycin on T7 DNA, RNA, and protein synthesis.** The effect of coumermycin on DNA, RNA, and protein synthesis during T7 infection of *E. coli* B/1 is shown in Fig. 2a, b, and c. The rate of both DNA and RNA synthesis was significantly reduced in the presence of the antibiotic. Protein synthesis was also reduced, but the effect was less pronounced and probably resulted from the inhibition of transcription.

To better measure the effect of coumermycin on phage RNA and protein synthesis, we also carried out this experiment using cells in which host synthesis was shut off prior to phage infection by irradiation with UV light (Fig. 2d, e, and f). It is apparent that phage transcription is significantly inhibited by the antibiotic, especially at later times during infection. Thus, at 6, 9, 12, and 15 min after infection coumermycin inhibited RNA synthesis by 47, 58, 63, and 81%, respectively.

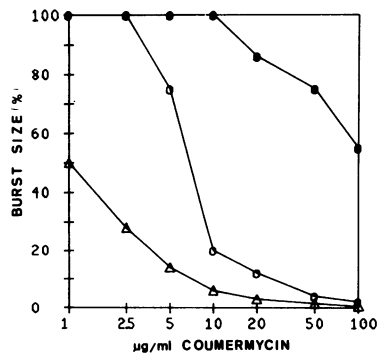


FIG. 1. *Coumermycin inhibits the growth of bacteriophage T7. One-step growth experiments were carried out in T broth at 30°C as described by Baird et al. (1). At 5 min after infection, infective centers were diluted into broth containing the indicated final concentrations of coumermycin. The burst size is presented as the percentage of a control containing no coumermycin. The bacterial strains used were: B/1 (△); NI 747 (○); NI 748 Cou$^r$ (●).*

To demonstrate that the effect of coumermycin on both T7 DNA and RNA synthesis results from inhibition of the *E. coli* DNA gyrase, we determined the effect of the antibiotic on phage DNA, RNA, and protein synthesis using the isogenic K-12 strains NI 747 Cou$^s$ and NI 748 Cou$^r$ (Fig. 3). The addition of 30 μg of coumermycin per ml to the resistant strain had little affect on T7 DNA, RNA, or protein synthesis (Fig. 3a, b, and c), whereas the coumermycin-sensitive K-12 strain gave results similar to those found for *E. coli* B/1 (Fig. 3e, f, and g). The experiments shown in Fig. 3 were done using UV-irradiated cells, but similar results have been obtained using non-irradiated cells.

The effects of coumermycin concentration on the inhibition of T7 DNA, RNA, and protein synthesis are compared in Fig. 4. The response to coumermycin was similar for the inhibition of both DNA and RNA synthesis, although in both cases synthesis was more sensitive to the antibiotic at later times during the infection.

In summary, coumermycin causes an almost complete inhibition of phage DNA replication and significantly reduces the rate of phage transcription. This strongly suggests that the *E. coli* DNA gyrase plays an important role in both T7 DNA and RNA synthesis. However, it is possible that DNA gyrase is not required for phage nucleic acid synthesis but that, in the presence of coumermycin, a drug-enzyme complex acts as an inhibitor. Although this second possibility seems unlikely, it cannot be ruled out at the present time.

**Transcription of early genes is not inhibited by coumermycin.** The results presented above indicate that at early times during infection T7 transcription is only slightly inhibited by coumermycin. To test directly whether early transcription is inhibited by the antibiotic, we measured the effect of coumermycin on transcription during infection with a T7 gene 1 amber mutant. The T7 gene 1 protein is an RNA polymerase that is required for transcription of the class II and class III genes. During infection by gene 1 mutants, only the class I or early genes are transcribed. In the absence of the gene 1 RNA polymerase, T7 transcription was completely unaffected by coumermycin (Fig. 5a). This suggests that DNA gyrase is not required for transcription of the early genes by the host RNA polymerase.

**Coumermycin inhibition of T7 transcription does not require DNA synthesis.** Since DNA gyrase plays an essential role in T7 DNA

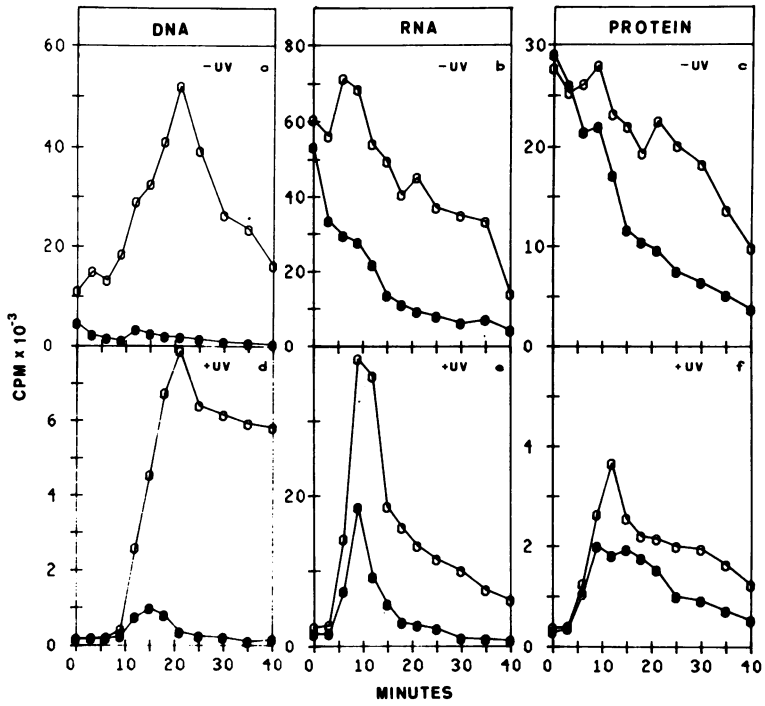


FIG. 2. *Effect of coumermycin on T7 DNA, RNA, and protein synthesis in normal and UV-irradiated cells. The rate of DNA (a, d), RNA (b, e), and protein (c, f) synthesis was measured at intervals after infection of normal (a, b, c) or UV-irradiated (d, e, f) E. coli B/1 with T7 phage as described in the text. Three minutes before infection, 30 μg of coumermycin per ml (●) or an equivalent amount of the drug diluent dimethyl sulfoxide (○) was added to the culture.*

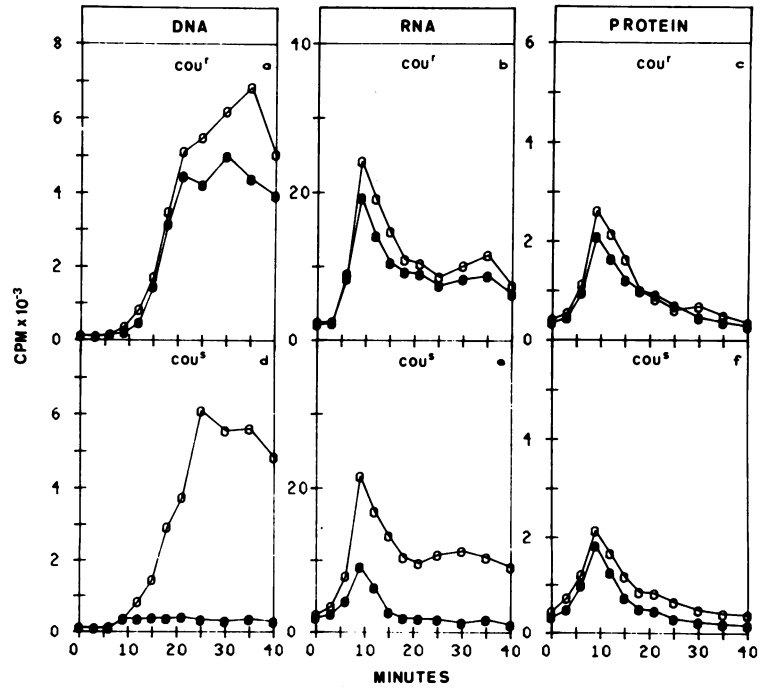


FIG. 3. *Effect of coumermycin on T7 DNA, RNA, and protein synthesis in coumermycin-sensitive and -resistant cells. Procedures were as described in the legend to Fig. 2. E. coli NI 748 Cou^r (a, b, c) and NI 747 Cou^s (d, e, g) were irradiated with UV light prior to infection. Control (○); 30 μg of coumermycin per ml (●).*

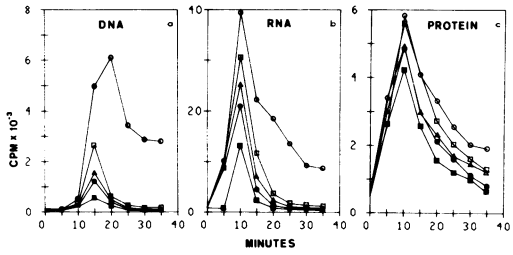


FIG. 4. *Effect of coumermycin concentration on T7 DNA, RNA, and protein synthesis. Procedures were as described in the legend to Fig. 2. E. coli B/1 was irradiated with UV light, and the indicated amounts of coumermycin were added 3 min prior to infection with T7 phage. Control* (○); *coumermycin at 3 μg/ml* (□); *10 μg/ml* (△); *30 μg/ml* (●); *100 μg/ml* (■).

replication, it seemed possible that the inhibition of late transcription by coumermycin might be a secondary effect resulting from the inhibition of DNA synthesis. Late transcription of T7 does not normally require DNA replication, but abortive replication in the absence of DNA gyrase might produce lesions in the DNA which inhibit transcription. It was therefore of interest to determine whether DNA synthesis is required for coumermycin inhibition of transcription. T7 provides an excellent system to test the requirement for DNA synthesis, since a number of phage mutants are defective in DNA replication but not in transcription. We measured the effect of coumermycin on phage transcription in cells infected with T7 gene 5 (DNA polymerase) and gene 6 (exonuclease) mutants. The T7 DNA polymerase is required for all stages of phage DNA replication. However, during infection with a T7 gene 5 mutant, the inhibition of transcription by coumermycin was identical to that observed during a wild-type infection (Fig. 5b). The T7 exonuclease is not required for early stages of phage DNA replication, but is apparently required for the formation of large replicative intermediates. As will be discussed later, structures containing up to several hundred phage equivalents of DNA (concatemers) are formed during replication in normal infections. Concatemers are not found in cells infected with T7 gene 6 mutants (12). However, the gene 6 mutant infection exhibited a normal coumermycin inhibition of both transcription (Fig. 5c) and DNA synthesis (data not shown). Thus, coumermycin inhibition of transcription does not require DNA synthesis, which suggests that DNA gyrase plays a direct role in phage RNA synthesis. These data also indicate that DNA gyrase is required for DNA and RNA synthesis even when large replicative intermediates are not formed and when the template DNA remains in unit-length linear molecules.

**Coumermycin selectively inhibits the synthesis of late proteins.** Since our studies on the kinetics of phage RNA synthesis suggested that late transcription is preferentially inhibited by coumermycin, it was of interest to determine the effect of the antibiotic on the patterns of protein synthesis during phage infection. Although protein synthesis is not as strongly inhibited by coumermycin as is DNA or RNA synthesis, it seemed possible that the pattern of protein synthesis might be altered by the drug. For example, the synthesis of functional late mRNA might be completely blocked by the antibiotic, and protein synthesis observed at late times in the presence of coumermycin could represent residual translation of early mRNA. Alternatively, the transcription of some late genes could be affected more strongly than transcription of others. To examine these possibilities, we labeled T7 proteins with $^{14}$C-amino acids at intervals during infection in the presence and absence of coumermycin. The proteins were then analyzed by electrophoresis in polyacrylamide gels containing sodium dodecyl sulfate (Fig. 6).

Synthesis of T7 early proteins, the products of genes which are transcribed by the *E. coli* RNA polymerase and are expressed immediately after infection (e.g., genes 0.7, 1, and 1.3), was not affected by coumermycin. This supports our previous conclusion that transcription of the early genes is insensitive to the drug. In contrast, synthesis of many of the late proteins was severely inhibited by coumermycin. However, not all of the genes transcribed by the T7 RNA polymerase were affected equally by the antibiotic. The expression of the class II genes 4, 5, 6, and HDP (helix destabilizing protein) appeared to be relatively insensitive to coumer-

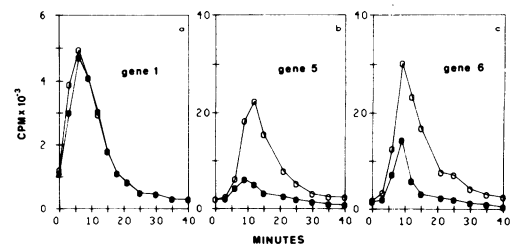


FIG. 5. *Effect of coumermycin on RNA synthesis in cells infected with T7 gene 1, gene 5, or gene 6 amber mutants. E. coli B/1 was irradiated with UV light and infected with T7 phage bearing an amber mutant in gene 1 (a), gene 5 (b), or gene 6 (c), and the rate of RNA synthesis was measured at intervals after infection as described in the text. Control* (○); *30 μg of coumermycin per ml* (●).

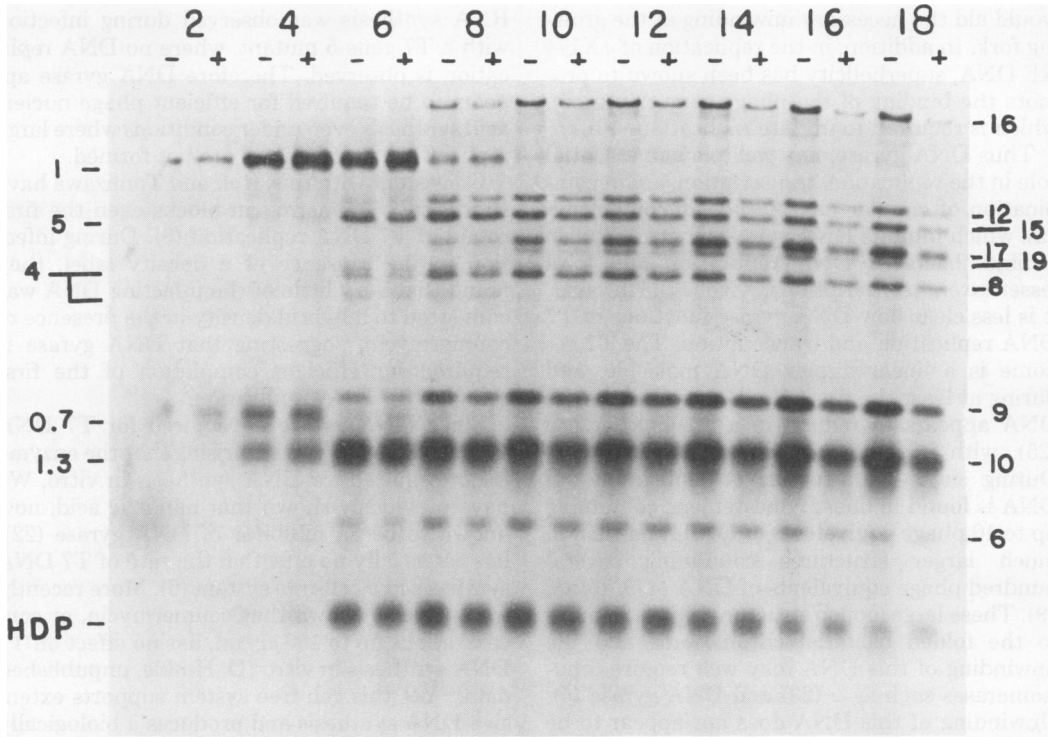


FIG. 6. *Polyacrylamide gel electrophoresis of T7 proteins synthesized in the presence or absence of coumermycin. Procedures were as described by Studier (20, 21). UV-irradiated E. coli B/1 was infected with T7 phage at a multiplicity of 10 in M9 medium at 30°C. At 2-min intervals 0.1-ml samples of the infected culture were added to 10 μl of a mixture containing $^{14}$C-amino acids (200 μCi/ml) and 1 mg of unlabeled arginine per ml. After 2 min at 30°C, 1 ml of T broth was added to each sample, and incubation continued for 4 min. The cells were then collected by centrifugation, suspended in 50 mM Tris (pH 6.8) containing 1% mercaptoethanol, 1% sodium dodecyl sulfate, and 10% glycerol, incubated for 2 min at 100°C, and subjected to electrophoresis in a discontinuous sodium dodecyl sulfate-containing buffer system on a 10% polyacrylamide slab gel. Radioactivity on the dried gel was visualized by autoradiography. The number at the top of each track is the time (minutes) after infection at which the pulse of $^{14}$C-amino acids began for the control culture (−) and a culture containing 50 μg of coumermycin per ml (+). The number of the T7 gene that specifies each protein as identified by Studier (20; personal communication) is given at the sides. The helix-destabilizing protein (HDP) has not been assigned a gene number.*

mycin, whereas the expression of genes near the right end of the T7 genome (for example, genes 12, 15, 16, 17, and 19) was severely inhibited.

Thus, T7 gene expression exhibits a gradient of sensitivity to the antibiotic coumermycin. The expression of early genes, at the extreme left of the T7 genome, is insensitive to the drug, while the expression of genes at the right end of the genome is severely inhibited. The expression of genes in the middle of the genome exhibits intermediate levels of inhibition.

## DISCUSSION

*E. coli* DNA gyrase is required in vivo to maintain a circular DNA in an underwound or negatively supercoiled state. This has been demonstrated for superinfecting phage lambda DNA (5) and more recently for the folded bacterial chromosome itself (3). It seems likely that the negative supercoiling of a circular DNA may provide an important driving force for DNA replication, transcription, and recombination. It has been shown that in vitro such DNA molecules can hybridize with single-strand DNA fragments to form the triple-stranded joint commonly postulated as an intermediate in recombination (7). Furthermore, site-specific recombination of phage lambda in vitro requires superhelical DNA (13). In fact, DNA gyrase was first identified on the basis of its role in this reaction (14). The transcription of a number of circular DNAs in vitro (2, 16, 24) has been shown to be enhanced by superhelicity. In replication, maintaining the DNA in an underwound state

would aid the necessary unwinding at the growing fork. In addition, in the replication of $\phi$X174 RF DNA, superhelicity has been shown to promote the binding of the phage gene A protein, which is required to initiate replication (10).

Thus DNA gyrase may well play an essential role in the replication, transcription, and recombination of circular DNAs. Indeed, coumermycin, which inhibits DNA gyrase in vitro (5), also inhibits bacterial DNA replication and to a lesser extent transcription in vivo (17). However, it is less clear how DNA gyrase functions in T7 DNA replication and transcription. The T7 genome is a linear duplex DNA molecule, and during at least the first round of replication the DNA appears to remain in a linear structure (25), with no obvious topological constraints. During subsequent rounds of replication the DNA is found in linear concatemers, containing up to 10 phage equivalents of DNA, and then in much larger structures containing several hundred phage equivalents of DNA (4, 9, 15, 18, 19). These large looped structures appear similar to the folded bacterial chromosome and the unwinding of this DNA may well require topoisomerases such as $\omega$ (23) and DNA gyrase (5). Unwinding of this DNA does not appear to be constrained in vitro, since the addition of ethidium bromide does not produce supercoiled DNA (15). However, it seems quite likely that in vivo these large replication structures contain topologically isolated DNA loops analogous to those found in the bacterial chromosome (25). Thus, at least during the later stages of phage growth it seems reasonable that phage DNA replication and transcription would be affected by the DNA gyrase inhibitor coumermycin. In fact, we find that phage nucleic acid synthesis is less sensitive to coumermycin at early times during infection than it is at later times when the large replicative intermediates are formed. This is particularly true of RNA synthesis, where only late transcription appears to be inhibited by coumermycin; but with DNA synthesis, which is more completely inhibited by the antibiotic, we also see a small amount of synthesis at early times (10 to 15 min) even at very high coumermycin concentrations (for example, Fig. 4). This suggests that a small amount of phage DNA replication can occur in the absence of DNA gyrase activity. However, the formation of large concatenated DNA intermediates is apparently not necessary for coumermycin inhibition of DNA and RNA synthesis. We found a normal inhibition of both DNA synthesis (data not shown) and RNA synthesis (Fig. 5) during infection with a T7 gene 6 amber mutant, although concatemers are apparently not formed during this infection (12). In addition, a normal inhibition of RNA synthesis was observed during infection with a T7 gene 5 mutant, where no DNA replication is observed. Therefore DNA gyrase appears to be required for efficient phage nucleic acid synthesis even under conditions where large replicative intermediates are not formed.

Consistent with this, Itoh and Tomizawa have shown that coumermycin blocks even the first round of T7 DNA replication (8). During infection in the presence of a density label, they found that very little of the infecting DNA was converted to a hybrid density in the presence of coumermycin, suggesting that DNA gyrase is required for efficient completion of the first round of T7 DNA replication.

Since DNA gyrase is required for T7 DNA synthesis in vivo, it is surprising that the enzyme is not required for DNA synthesis in vitro. We have previously shown that nalidixic acid, now known to be an inhibitor of DNA gyrase (22), has essentially no effect on the rate of T7 DNA synthesis in a cell-free system (6). More recently we have also shown that coumermycin, at concentrations up to 100 $\mu$g/ml, has no effect on T7 DNA synthesis in vitro (D. Hinkle, unpublished data). Yet this cell-free system supports extensive DNA synthesis and produces a biologically active product (11). Perhaps attachment of the DNA to the cell membrane and/or polysomes in vivo produces topological constraints that are not present in vitro. This may also explain why DNA gyrase is required for efficient transcription of the late, but not the early, T7 genes, even in the absence of DNA synthesis. Immediately after infection the DNA is presumably relatively free of polyribosomes and other constraints, so that the limited unwinding of the DNA helix, which takes place during the binding of RNA polymerase to the promoter and the subsequent RNA synthesis, can occur in the absence of DNA gyrase activity. As the infection proceeds, the constraints on the unwinding of the DNA helix may become more severe, resulting in the observed gradient of coumermycin inhibition of T7 protein synthesis (Fig. 6). Alternatively, the T7 RNA polymerase may bind to and unwind some T7 promoters more easily than others, so that the negative torque provided by DNA gyrase stimulates the transcription of some genes more than others.

It will be interesting to determine whether DNA gyrase affects the pattern of transcription by the purified T7 RNA polymerase. In addition, as systems are developed for replication of T7 DNA using purified proteins, the effect of DNA gyrase on replication can be tested directly.

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