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Effect of Rifampin on the Growth of Bacteriophage T5

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A rifampin-resistant mutant of *Escherichia coli* F with an altered ribonucleic acid (RNA) polymerase was isolated and was shown to support the growth of phage T5 in the presence of rifampin. In contrast, wild-type, rifampin-sensitive cells of *E. coli* F did not support the growth of T5 in the presence of rifampin. We concluded, therefore, that no phage-specific RNA polymerase is essential to the development of phage T5. Rather, the host RNA polymerase, or at least that portion of the host RNA polymerase that is responsible for rifampin sensitivity, is required for the transcription of all essential regions of the T5 deoxyribonucleic acid. These conclusions are supported by in vitro measurements of the rifampin sensitivity of the RNA polymerase activities extracted from infected and uninfected cells. The rifampin sensitivity of the RNA polymerase activity extracted from uninfected cells was similar to the rifampin sensitivity of the RNA polymerase activity extracted 30 min after infection.

After complete transfer of the T5 genome into host cells, three classes of phage-specific ribonucleic acid (RNA) are sequentially induced (25). The synthesis of pre-early (class I) RNA is followed in sequence by the synthesis of early (class II) and then late (class III) RNA. The rate of synthesis of each class of RNA in rifampin-sensitive bacteria infected with phage T5, as well as the rate of RNA synthesis in uninfected rifampin-sensitive bacteria, has been shown to be completely inhibited by rifampin (26). Since rifampin acts directly on the deoxyribonucleic acid (DNA)-dependent RNA polymerase of *Escherichia coli* (6), these results suggest that the host RNA polymerase, or at least that portion of the host RNA polymerase that is sensitive to rifampin, is required for the transcription of pre-early, early, and late genes. However, these results do not preclude the possibility that an RNA polymerase which is sensitive to rifampin is induced by phage T5 and is required for the transcription of a certain region(s) of the T5 DNA. Experiments presented in this report support the conclusion that the de novo synthesis of a phage-specific RNA polymerase is not a requirement for the transcription of essential regions of the T5 genome.

MATERIALS AND METHODS

Bacteria and bacteriophage. Wild-type T5 was grown in *E. coli* F, a fast-adsorbing host for T5, and was purified by differential centrifugation. *E. coli* F and a strain of *E. coli* F resistant to rifampin were used for these experiments.

The rifampin-resistant mutant was isolated without mutagenesis as described by Haselkorn et al. (7) after ethylenediaminetetraacetic acid (EDTA) treatment as described by Leive (11). Essentially, this consisted of treating a culture of *E. coli* F with EDTA just before plating, via the overlay method as described by Adams (1), on soft agar containing 400 μg of rifampin per ml. About 1 cell in 10^8 formed a colony under these conditions. One such colony was picked and grown to log phase in nutrient broth containing 400 μg of rifampin per ml; the selection procedure, but not the EDTA treatment, was repeated. A single colony was picked and used as the source of rifampin-resistant cells. The growth rate of the rifampin-resistant mutant in the absence of rifampin was identical to that of the wild type. However, in the presence of 400 μg of rifampin per ml, the generation time of the rifampin-resistant mutant was about 50% longer than that of the wild type grown in the absence of rifampin.

Media. Cells were grown in nutrient broth prepared according to Adams (1) and adjusted to *p*H 7.2 with NaOH. Plating and soft agar contained 1.1 and 0.7% dehydrated agar (Difco), respectively, in nutrient broth. The composition of M-9 adsorption buffer (basically M-9 medium that lacks utilizable sources of carbon and nitrogen) has been described previously (4). All liquid or plating media used for the adsorption or growth of phage contained 6×10^{-4} M $CaCl_2$.

Preparation of crude extracts. Cells were grown to 4×10^8 per ml, harvested by centrifugation, and resuspended in either (i) 0.01 M tris(hydroxymethyl)-

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aminomethane (Tris)-hydrochloride buffer (*p*H 8.0), containing 0.01 M $MgCl_2$ and 0.0001 M EDTA, at a concentration of 5×10^{10} per ml or (ii) M-9 adsorption buffer at a concentration of 5×10^9 per ml. Cells in M-9 adsorption buffer were then infected at an input ratio of approximately five phages per cell. After incubation for 15 min at 37 C for the adsorption of phage, the phage-bacterium complexes were diluted 10-fold into nutrient broth, and aeration was begun. After incubation at 37 C for 30 min, the infected cells were poured into a centrifuge bottle containing 0.5 g of cracked ice per ml of culture, harvested, and resuspended in 0.01 M Tris-hydrochloride buffer (*p*H 8.0), containing 0.01 M $MgCl_2$ and 0.0001 M EDTA, at a concentration of 5×10^{10} per ml. Infected and uninfected cells were then sonically treated. Residual whole cells and cell debris were removed by centrifugation at 5,860 × *g* for 10 min, and ribosomes were removed from the resulting supernatant fluid by centrifugation at 109,000 × *g* for 1 hr. All steps in the preparation of the crude extracts were carried out at 4 C. Protein concentrations were determined by the method of Lowry et al. (13).

Assay for RNA polymerase. RNA polymerase activity was assayed by a slight modification of the method of Chamberlin and Berg (5). The reaction mixture (final volume, 0.125 ml), with calf thymus DNA as exogenous template, contained 5 μmoles of Tris-hydrochloride buffer (*p*H 8.0), 0.5 μmole of $MgCl_2$, 50 nmoles each of adenosine, guanosine, and uridine triphosphate (ATP, GTP, and UTP), 5 nmoles and 120,000 counts/min of ^{14}C-cytidine triphosphate (CTP), 1.5 μmoles of 2-mercaptoethanol, 40 nmoles of calf thymus DNA, and 55 to 234 μg of protein. The reaction was initiated by the addition of nucleoside triphosphates, and samples were incubated for 4 min at 30 C. For the measurement of the synthesis of poly r(A-U), the reaction mixture was as above except that: poly d(A-T) was substituted for calf thymus DNA; GTP and UTP were omitted; ^{14}C-UTP replaced ^{14}C-CTP; the reaction was initiated by the addition of a mixture of poly d(A-T) and nucleoside triphosphates; incubation was for 10 min; and the reaction mixture contained 35 to 39 μg of protein. Acid insoluble-radioactivity was determined by the method of Mans and Novelli (16).

Determination of the effect of the time of rifampin addition. Cells were grown to 2×10^8 per ml, harvested by centrifugation, resuspended in M-9 adsorption buffer at a concentration of 5×10^9 per ml, and infected with an equal volume of phage at an input ratio of 0.1 phage per cell. After incubation for 15 min at 37 C for the adsorption of phage, the infected culture was diluted 10^5-fold into nutrient broth and aeration was begun. After incubation at 37 C for the time indicated in Table 3, rifampin was added to a final concentration of 400 μg per ml, and aeration was continued so that the total time of incubation after dilution was 30 min. At this time, a 0.1-ml sample was removed and plated via the overlay method (1) with two drops of an overnight culture of rifampin-resistant *E. coli* F as indicator bacteria. For plating, the soft agar contained rifampin at a concentration of 400 μg per ml except when rifampin-sensitive cells were infected and incubated without rifampin as a control.

One-step growth experiments. One-step growth experiments were performed essentially according to Adams (1). Cells were grown to 2×10^8 per ml, harvested by centrifugation, resuspended in M-9 adsorption buffer at a concentration of 5×10^9 per ml, and infected with phage at an input ratio of 0.1 phage per cell. After incubation for 15 min at 37 C for the adsorption of phage, the infected culture was diluted at least 10^5-fold into nutrient broth at 37 C (time = 0 min in Fig. 4 and Fig. 5) and aeration was begun. At various times after dilution, 0.1-ml samples were withdrawn and plated with two drops of an overnight culture of indicator bacteria via the overlay method (1). The indicator bacteria were either the rifampin-sensitive strain of *E. coli* F or the rifampin-resistant strain of *E. coli* F depending on which bacterial strain was used as host for the one-step growth experiment. For the one-step growth experiment in the presence of rifampin, adsorption buffer and nutrient broth contained rifampin at a concentration of 400 μg per ml. Thus, rifampin was present throughout the adsorption period as well as throughout the phage growth cycle.

Determination of the level of unadsorbed and intracellular phage. For the measurement of unadsorbed and intracellular phage in the presence of rifampin, the cells were grown to 2×10^8 per ml, harvested by centrifugation, and resuspended in nutrient broth, containing 400 μg of rifampin per ml, at a concentration of 5×10^9 per ml. The cells were infected at 37 C (time = 0 min in Fig. 5) at an input ratio of 0.1 phage per cell and aeration was begun. At various times after infection, 0.1-ml samples were withdrawn into 0.9 ml of nutrient broth to which seven to eight drops of chloroform had been added. After vigorous shaking, the aqueous layer was diluted and plated via the overlay method (1) with two drops of an overnight culture of rifampin-sensitive *E. coli* F as indicator bacteria. Because most of the rifampin partitions with the chloroform, the growth of the indicator bacteria was not inhibited.

Chemicals. Actinomycin D was a gift from Merck & Co., Inc., and rifampin [3-(4-methyl-piperazinyl-iminomethyl)rifamycin SV] was purchased from Mann Research Laboratories. Tris was purchased from Sigma Chemical Co.; 2-mercaptoethanol, from Eastman Organic Chemicals; calf thymus DNA, from Worthington Biochemical Corp.; and poly d(A-T), from Miles Laboratories. ^{14}C-CTP and ^{14}C-UTP were purchased from Schwarz BioResearch, and unlabeled nucleoside triphosphates were purchased from P-L Biochemicals.

RESULTS

Effect of rifampin on the RNA polymerase activity in crude extracts prepared from rifampin-sensitive and -resistant cells of E. coli F. Extracts of rifampin-sensitive and rifampin-resistant cells of *E. coli* F were assayed for RNA polymerase activity as described in Materials and Methods. For extracts prepared from both rifampin-sensi-

tive and rifampin-resistant cells, the rate of incorporation of ^{14}C-CTP or ^{14}C-UTP into acid-insoluble products was linear with respect to time and with respect to protein concentration. The RNA polymerases from rifampin-sensitive and rifampin-resistant cells were both inhibited by actinomycin D (Fig. 1). Since actinomycin D inhibits DNA-directed RNA synthesis by binding to the DNA template (21), the RNA polymerase activities, in both extracts, must be DNA-dependent.

To determine whether the ability of the rifampin-resistant cells to grow in the presence of rifampin is due to an altered RNA polymerase, rather than an altered permeability to rifampin, the sensitivity to rifampin of the RNA polymerase from rifampin-sensitive and rifampin-resistant cells was measured. With calf thymus DNA as exogenous template (Fig. 2), the RNA polymerase from rifampin-sensitive cells was 80% inhibited by 12 μg of rifampin per ml, but the RNA polymerase from rifampin-resistant cells was inhibited only slightly (about 9%) by this concentration of rifampin. In effect, 20% of the RNA polymerase activity in the extract prepared from rifampin-sensitive cells, with calf thymus DNA as exogenous template, was insensitive to rifampin. This activity was likely due to RNA

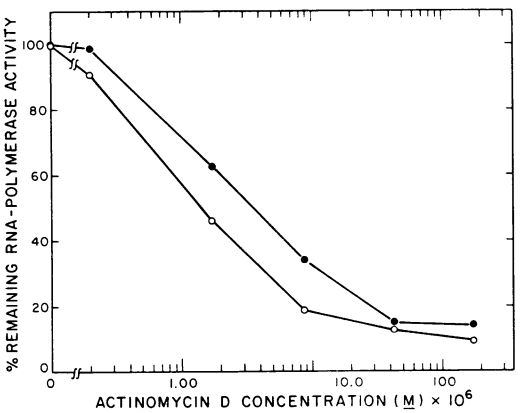

FIG. 1. *Effect of actinomycin D on the RNA polymerase activity, with calf thymus DNA as template, in crude extracts of rifampin-sensitive (●) and rifampin-resistant (○) cells of E. coli F. Experimental details are given in Materials and Methods. The percentages of remaining RNA polymerase activity with different concentrations of actinomycin D were calculated from the amount of ^{14}C-CTP incorporated as compared with the controls without actinomycin D. The percentages of remaining RNA polymerase activity of the controls without actinomycin D were taken as 100%. For the rifampin-sensitive and rifampin-resistant extracts, these values were 0.29 and 0.63 nmole of ^{14}C-CTP incorporated/mg of protein, respectively.*

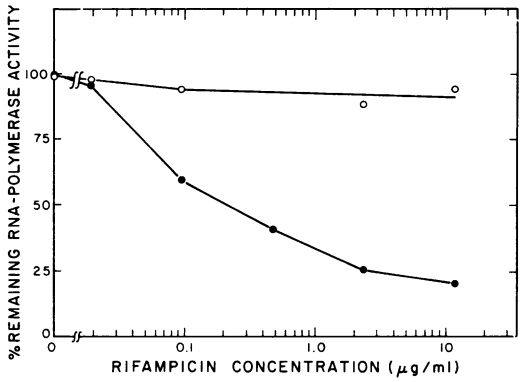

FIG. 2. *Effect of rifampin (formerly called rifampicin) on the RNA polymerase activity, with calf thymus DNA as template, in crude extracts of rifampin-sensitive (●) and rifampin-resistant (○) cells of E. coli F. The order of addition of reaction components was: extract, calf thymus DNA, rifampin, nucleoside triphosphates. Additional experimental details are given in Materials and Methods. The percentages of remaining RNA polymerase activity with different concentrations of rifampin were calculated from the amount of ^{14}C-CTP incorporated as compared with the controls without rifampin. The percentages of remaining RNA polymerase activity of the controls without rifampin were taken as 100%. For the rifampin-sensitive and rifampin-resistant extracts, these values were 0.30 and 0.58 nmole of ^{14}C-CTP incorporated/mg of protein respectively.*

chain elongation, upon the addition of nucleoside triphosphates, by endogenous DNA-bound polymerase that had initiated the synthesis of RNA chains prior to the addition of rifampin. Rifampin inhibits only the initiation of RNA chains and has no effect on RNA chain elongation (24). In addition, the binding of polymerase to endogenous as well as calf thymus DNA before the addition of rifampin will decrease the sensitivity of RNA chain initiation to inhibition by rifampin. DNA-bound polymerase has been shown to be less sensitive to rifampin inhibition than unbound polymerase (2, 12). Indeed, preincubation of the rifampin-sensitive extract with 0.24 μg of rifampin per ml prior to the addition of poly d(A-T) as template inhibited ^{14}C-UTP incorporation by 97% (Fig. 3).

To eliminate the possibility that the rifampin-resistant cells inactivate rifampin, the RNA polymerase activity in the extract from rifampin-sensitive cells was measured in the presence of rifampin and an extract from rifampin-resistant cells. The data in Table 1 show that the rifampin-sensitive RNA polymerase retained its sensitivity to rifampin even in the presence of the extract from rifampin-resistant cells.

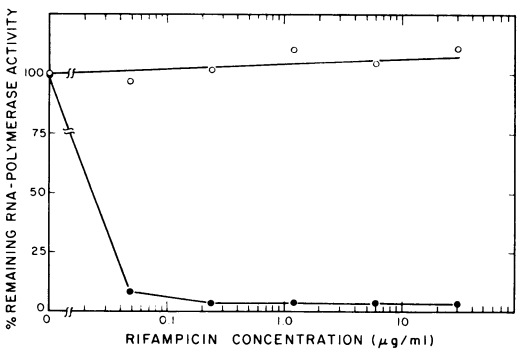

FIG. 3. *Effect of rifampin (formerly called rifampicin) on the RNA polymerase activity, with poly d(A-T) as template, in crude extracts of rifampin-sensitive (●) and rifampin-resistant (○) cells of E. coli F. The extracts were preincubated with the indicated concentration of rifampin for 10 min before the addition of poly d(A-T) and nucleoside triphosphates. Additional experimental details are given in Materials and Methods. The percentages of remaining RNA polymerase activity with different concentrations of rifampin were calculated from the amount of ^{14}C-UTP incorporated as compared with the controls without rifampin. The percentages of remaining RNA polymerase activity of the controls without rifampin were taken as 100%. For the rifampin-sensitive and rifampin-resistant extracts, these values were 1.42 and 1.43 nmoles of ^{14}C-UTP incorporated/mg of protein, respectively.*

TABLE 1. *Stability of rifampin in the presence of crude extract from rifampin-resistant cells of E. coli F*

Extract (mg of protein) from		Rifampin (μg)	^{14}C-CTP incorporated in 4 min at 30 C (nmoles)
Rifampin-sensitive cells	Rifampin-resistant cells		
0.112	—	—	0.056
—	0.055	—	0.053
0.112	0.055	—	0.098
0.112	0.055	0.30	0.061

From these results, we conclude that the observed difference in sensitivity to rifampin between the rifampin-sensitive and rifampin-resistant RNA polymerase is due to an actual structural difference between the two enzymes. This structural difference accounts for the ability of rifampin-resistant cells to grow in the presence of rifampin.

Effect of rifampin on the growth of T5$^+$ in rifampin-sensitive and -resistant cells of E. coli F. The latent period and burst size of wild-type T5 were determined in rifampin-resistant cells in the presence and absence of rifampin (Fig. 4) and in rifampin-sensitive cells in the absence of rifampin (Fig. 5) via one-step growth experiments. We found that the rifampin-sensitive infective centers could not be measured after exposure to 400 μg of rifampin per ml, apparently because the RNA polymerase from these cells does not recover sufficiently from rifampin treatment (even after 10^6-fold dilution) during the period of logarithmic growth of the bacterial lawn to allow the formation of plaques. Therefore, the inhibition of the growth of T5$^+$ in rifampin-sensitive cells in the presence of rifampin was demonstrated by measuring the level of unadsorbed and intracellular phage at 10-min intervals throughout a 90-min period after phage infection (Fig. 5). The results of these experiments are summarized in Table 2.

Phage T5 production in the wild-type, rifampin-sensitive host was completely inhibited by 400 μg of rifampin per ml. After 90 min, only 0.01% of the infecting phages could be measured in the aqueous layer after chloroform treatment. In contrast, phage production in the rifampin-resistant host was only slightly inhibited by 400 μg of rifampin per ml. The minimal latent period of T5 in rifampin-sensitive and in rifampin-resistant cells was virtually the same and, in addition,

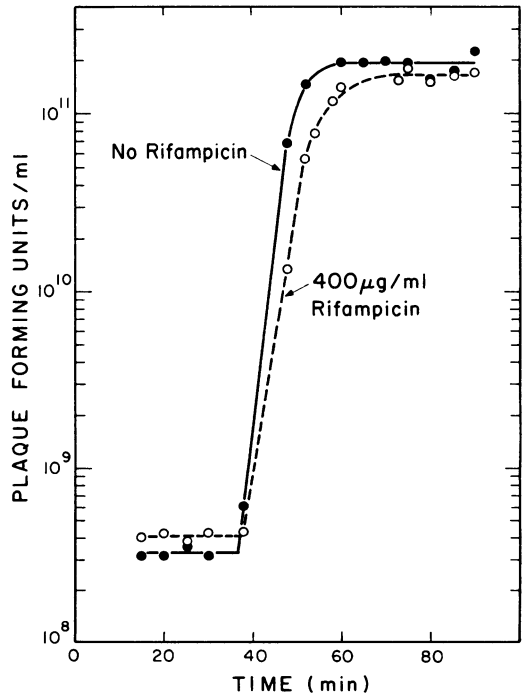

FIG. 4. *One-step growth curves of T5$^+$ in the presence and absence of rifampin (formerly called rifampicin) with rifampin-resistant cells of E. coli F as host. Experimental details are given in Materials and Methods.*

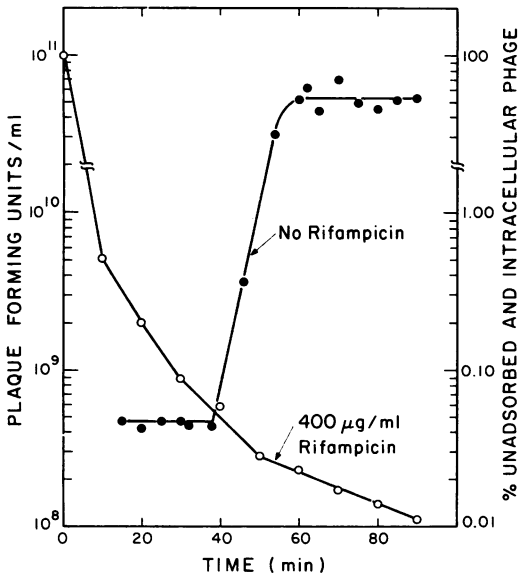

FIG. 5. *One-step growth curve of T5+ in the absence of rifampin (formerly called rifampicin) (●) and the rate of disappearance of unadsorbed T5+ in the presence of rifampin (○) with rifampin-sensitive cells of E. coli F as host. Experimental details are given in Materials and Methods.*

TABLE 2. *Effect of rifampin on the growth of T5+ in rifampin-sensitive and rifampin-resistant cells of E. coli F*

Host bacteria	No rifampin		Rifampin, 400 μg/ml	
	Avg burst size	Minimal latent period (min)	Avg burst size	Minimal latent period (min)
Rifampin-sensitive	120	38	0[a]	—
Rifampin-resistant	580	37	400	38

[a] See text and Fig. 5.

rifampin had no effect on the minimal latent period of T5 when rifampin-resistant cells were used as host.

Effect of the time of addition of rifampin during the T5 latent period. To determine whether the inhibition by rifampin of the growth of T5 in rifampin-sensitive cells was due to the inhibition of the synthesis of a phage-specific RNA polymerase, the effect of the time of addition of rifampin during the T5 latent period was studied. The data in Table 3 show that the complete inhibition of the growth of T5 in rifampin-sensitive cells could be achieved by the addition of rifampin 10 min after infection. Since the synthesis of pre-early proteins begins about 1 min after infection and the synthesis of early proteins begins about 5 min after infection (14), the inhibition of the growth of T5 in rifampin-sensitive cells cannot be due to the inhibition of the synthesis of a stable phage-specific RNA polymerase. In addition, the inhibition of the growth of T5 in rifampin-sensitive cells observed upon the addition of rifampin 10 min after infection cannot be due to the inhibition of the synthesis of an unstable, early, phage-specific RNA polymerase, because Pispa et al. (20) have shown that the addition of chloramphenicol to infected cells 8 min after infection does not prevent the appearance of late RNA.

Table 3 shows that the complete inhibition of the growth of T5 in rifampin-sensitive cells cannot be achieved by the addition of rifampin 20 or 30 min after infection. Sirbasku and Buchanan (26) reported that the inhibition of RNA synthesis in rifampin-sensitive cells infected with phage T5 is slower if rifampin is added 20 min after infection than if rifampin is added 4 or 8 min after infection. The slower response to rifampin of RNA synthesis when rifampin is added 20 min after infection may reflect changes in the permeability of the cell to rifampin during the latent period or differences in the stability of late RNA with respect to the stability of pre-early or early RNA. Our observation that the further growth of T5 cannot be inhibited by the addition of rifampin 20 min after infection may also be a reflection of permeability changes, but is probably also due to a reduced requirement for late RNA synthesis, because late RNA synthesis begins about 9 min after infection (25) and, therefore, all species of late RNA are presumably present by 20 min after infection.

Effect of rifampin on the RNA polymerase activity in crude extracts prepared from infected and

TABLE 3. *Effect of the time of addition of rifampin during the T5 latent period on the further growth of T5+*

Time of rifampin addition (min)	Infective centers (as % of initial value)[a]	
	Rifampin-sensitive host	Rifampin-resistant host
0	0	90
10	0	—
20	90	—
30	107	—
No rifampin	102	—

[a] The plaque counts were corrected for dilution and compared with the infective centers measured in M-9 adsorption buffer before dilution into nutrient broth (see Materials and Methods).

uninfected, rifampin-sensitive and -resistant cells of E. coli F. Although the rate of RNA synthesis in rifampin-sensitive cells infected with phage T5 has been shown to be sensitive to inhibition by rifampin throughout the T5 latent period, the inhibition of RNA synthesis is slower if rifampin is added during the period of synthesis of late RNA (25, 26). Therefore, the possibility existed that a phage-specific RNA polymerase is required for the transcription of late genes and that this enzyme is somewhat more resistant to rifampin than the RNA-polymerase present in uninfected, rifampin-sensitive cells. To test this possibility, the time course of ^{14}C-CTP incorporation in the presence and absence of rifampin was followed by use of extracts prepared from uninfected, rifampin-sensitive and rifampin-resistant cells as well as extracts prepared from rifampin-sensitive and rifampin-resistant cells 30 min after infection. At this time, the infected cells are actively synthesizing late RNA (25). Figure 6 shows that the RNA polymerase extracted from rifampin-sensitive cells 30 min after infection was slightly *less* resistant to rifampin than the RNA polymerase extracted from uninfected, rifampin-sensitive cells. In addition, the rifampin sensitivity of the RNA polymerase extracted from rifampin-resistant cells 30 min after infection was virtually identical to the rifampin sensitivity of the RNA-polymerase extracted from uninfected, rifampin-resistant cells (Fig. 7).

The results presented in this report establish that the rifampin sensitivity of T5 development depends on the rifampin sensitivity of the host RNA polymerase. If the host RNA polymerase is insensitive to rifampin, the growth of T5 is insensitive to rifampin. However, if the host RNA-polymerase is sensitive to rifampin, the growth of T5 is sensitive to rifampin, and this sensitivity can be demonstrated 9 min after the appearance of pre-early proteins and 5 min after the appearance of early proteins, even though the synthesis of late RNA is not sensitive to inhibition by chloramphenicol by 8 min after infection (20). Therefore, that portion of the host RNA polymerase that is responsible for rifampin sensitivity must be required for the transcription of all essential regions of the T5 DNA.

DISCUSSION

Three groups of phage-specific proteins are sequentially induced after infection by phage T5 (14). Pre-early proteins are followed in sequence by early and then late proteins. The synthesis of

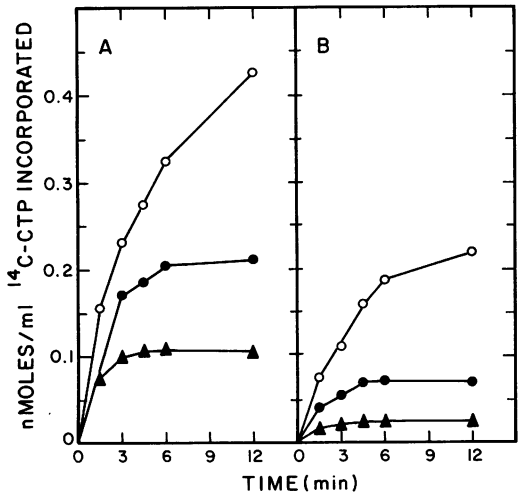

FIG. 6. *Effect of rifampin on the RNA polymerase activity, with calf thymus DNA as template, extracted from (A) uninfected rifampin-sensitive cells and (B) rifampin-sensitive cells 30 min after infection with T5$^+$. (○) No rifampin; (●) 0.10 μg of rifampin per ml; (▲) 12 μg of rifampin per ml. The reaction mixture contained 100 μg of protein in A and 270 μg of protein in B. In both A and B, the reaction mixture contained 2.6 nmoles and 174,000 counts/min of ^{14}C-CTP. The order of addition of reaction components was the same as in Fig. 2. Additional experimental details are given in Materials and Methods.*

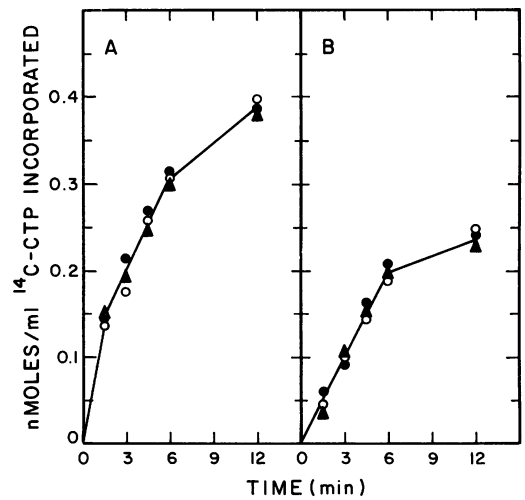

FIG. 7. *Effect of rifampin on the RNA polymerase activity, with calf thymus DNA as template, extracted from (A) uninfected rifampin-resistant cells and (B) rifampin-resistant cells 30 min after infection with T5$^+$. (○) No rifampin; (●) 0.10 μg of rifampin per ml; (▲) 12 μg of rifampin per ml. The reaction mixture contained 100 μg of protein in A and 210 μg of protein in B. In both A and B, the reaction mixture contained 2.6 nmoles and 174,000 counts/min of ^{14}C-CTP. The order of addition of reaction components was the same as in Fig. 2. Additional experimental details are given in Materials and Methods.*

these three groups of proteins is controlled by the synthesis of three corresponding groups of phage-specific RNA (25). Sirbasku and Buchanan (26) showed that rifampin completely inhibits the synthesis of phage-specific RNA in rifampin-sensitive bacteria infected with phage T5. However, although complete inhibition of RNA synthesis can be achieved by the addition of rifampin 4, 8, or 20 min after infection, the response to rifampin is slower if rifampin is added 20 min after infection than if rifampin is added 4 or 8 min after infection. In vitro measurements, presented in this report, of the rifampin sensitivity of the RNA polymerase activity extracted from infected and uninfected, rifampin-sensitive and rifampin-resistant cells indicate that this is not due to the synthesis of a T5-specific RNA polymerase that is more resistant to rifampin than the RNA polymerase present in uninfected, rifampin-sensitive cells. These results indicate that no phage-specific RNA polymerase that is resistant to rifampin is induced by phage T5. However, these results do not eliminate the possibility that a rifampin-sensitive RNA polymerase is induced by T5. We have shown in this report, however, that the growth of T5 in rifampin-resistant bacteria is insensitive to rifampin, and that the development of T5 in rifampin-sensitive bacteria can be arrested by the addition of rifampin 9 min after the synthesis of pre-early proteins has begun and 5 min after the synthesis of early proteins has begun. In this regard, Pispa et al. (20) showed that the addition of chloramphenicol 8 min after infection does not prevent the appearance of late RNA, so that the sensitivity to rifampin inhibition of T5 development during the period of early protein synthesis does not reflect the inhibition of the synthesis of an unstable, early, T5-specific RNA polymerase. Therefore, no phage-specific RNA polymerase that is sensitive to rifampin can be required for the transcription of any essential region of the T5 DNA. Since the de novo synthesis of a T5-specific RNA polymerase is not a requirement for the transcription of pre-early, early, or late genes, the temporal separation of the three groups of proteins must involve some mechanism which does not involve the synthesis of a phage-specific RNA polymerase.

T5 has a unique mode of transfer of DNA to host cells. First, the initial 8% section of the phage DNA, which codes for the pre-early proteins, is transferred. Then, after the complete expression of two pre-early genes (A1 and A2), the remaining 92% of the phage DNA is transferred (4, 8, 10, 15). Thus, T5 has a built-in mechanism for the temporal separation of pre-early from early and late proteins. That is, transcription of early and late genes cannot begin until after the complete expression of pre-early genes A1 and A2. It would not be surprising, therefore, if the early genes of T5 were transcribed in vivo by the unmodified host RNA polymerase. Indeed, Bautz et al. (3) showed that the unmodified host RNA polymerase has the same capacity to transcribe early genes as pre-early genes in vitro.

The temporal separation of early and late proteins, however, must involve a mechanism that is not dependent on interrupted transfer of the phage DNA. Early and late genes enter the cell in a noninterrupted manner with the remaining 92% of the phage DNA (9). However, Pispa and Buchanan (19), as well as Yuan and Bremer (*personal communication*), have shown that the RNA polymerase from uninfected cells has a lowered capacity to transcribe late genes in vitro. In addition, the synthesis of at least one early protein has been shown to be a requirement for the synthesis of late RNA (17). Therefore, it seems reasonable to speculate that the temporal separation of early and late proteins might involve a modification of the host RNA polymerase by an early phage function to a form with an enhanced capacity to synthesize late RNA. This modifying phage function could be either a sigma or some other protein factor which modifies the host RNA polymerase by binding to the enzyme or a factor which modifies one or more of the existing subunits of the host RNA polymerase. In this regard, the α, β, and β' subunits of the host RNA polymerase have been shown to be present in a modified form after infection by phage T4 (27–29).

Alternatively, this early phage protein, required for the transcription of late genes, might alter the initiation specificity of the host RNA polymerase by binding to the T5 DNA at or near the promoter site(s) for the late operon(s). The isolation of a DNA-binding protein responsible for the activation of the *lac* operon of *E. coli* (22, 30) has recently been reported. In vivo studies indicate that this protein stimulates the transcription of the genes of the *lac* operon by binding to the *lac* promoter (18, 23).

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LITERATURE CITED

1. Adams, M. H. 1959. Bacteriophages. Interscience Publishers, Inc., New York.

2. Bautz, E. K. F., and F. A. Bautz. 1970. Initiation of RNA synthesis: the function of σ in the binding of RNA polymerase to promoter sites. Nature (London) **226**:1219–1222.
3. Bautz, E. K. F., J. J. Dunn, F. A. Bautz, D. A. Schmidt, and A. J. Mazaitis. 1970. Initiation and regulation of transcription by RNA polymerase. Proc. 1st Int. Lepetit Colloq. on RNA-Polymerase and Transcription, p. 90–109. North-Holland Publishing Co., Amsterdam.
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. Chamberlin, M., and P. Berg. 1962. Deoxyribonucleic acid-directed synthesis of ribonucleic acid by an enzyme from *Escherichia coli*. Proc. Nat. Acad. Sci. U.S.A. **48**:81–94.
6. Hartmann, G., K. O. Honikel, F. Knüsel, and J. Nüesch. 1967. The specific inhibition of the DNA-directed RNA synthesis by rifamycin. Biochim. Biophys. Acta **145**:843–844.
7. Haselkorn, R., M. Vogel, and R. D. Brown. 1969. Conservation of the rifamycin sensitivity of transcription during T4 development. Nature (London) **221**:836–838.
8. Hendrickson, H. E., and D. J. McCorquodale. 1971. Genetic and physiological studies of bacteriophage T5. I. An expanded genetic map of T5. J. Virol. **7**:612–618.
9. Lanni, Y. T. 1960. Invasion by bacteriophage T5. II. Dissociation of calcium-independent and calcium-dependent processes. Virology **10**:514–529.
10. Lanni, Y. T. 1969. Functions of two genes in the first-step-transfer DNA of bacteriophage T5. J. Mol. Biol. **44**:173–183.
11. Leive, L. 1965. RNA degradation and the assembly of ribosomes in actinomycin-treated *Escherichia coli*. J. Mol. Biol. **13**:862–875.
12. Lill, H., U. Lill, A. Sippel, and G. Hartmann. 1970. The inhibition of the RNA polymerase reaction by rifampicin. Proc. 1st Int. Lepetit Colloq. on RNA-Polymerase and Transcription, p. 55–64. North-Holland Publishing Co., Amsterdam.
13. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
14. McCorquodale, D. J., and J. M. Buchanan. 1968. Patterns of protein synthesis in T5-infected *Escherichia coli*. J. Biol. Chem. **243**:2550–2559.
15. McCorquodale, D. J., and Y. T. Lanni. 1970. Patterns of protein synthesis in *Escherichia coli* infected by amber mutants in the first-step-transfer DNA of T5. J. Mol. Biol. **48**:133–143.
16. Mans, R. J., and G. D. Novelli. 1961. Measurement of the incorporation of radioactive amino acids into protein by a filter-paper disk method. Arch. Biochem. Biophys. **94**:48–53.
17. Moyer, R. W., and J. M. Buchanan. 1969. Patterns of RNA synthesis in T5-infected cells. I. As studied by the technique of DNA-RNA hybridization-competition. Proc. Nat. Acad. Sci. U.S.A. **64**:1249–1256.
18. Pastan, I., and R. L. Perlman. 1968. The role of the *lac* promoter locus in the regulation of β-galactosidase synthesis by cyclic 3′5′-adenosine monophosphate. Proc. Nat. Acad. Sci. U.S.A. **61**:1336–1342.
19. Pispa, J. P., and J. M. Buchanan. 1971. Synthesis of bacteriophage T5 specific RNA *in vitro*. Biochim. Biophys. Acta **247**:181–184.
20. Pispa, J. P., D. A. Sirbasku, and J. M. Buchanan. 1971. Patterns of ribonucleic acid synthesis in T5-infected *Escherichia coli*. IV. Examination of the role of deoxyribonucleic acid replication. J. Biol. Chem. **246**:1658–1664.
21. Reich, E., R. M. Franklin, A. J. Shatkin, and E. L. Tatum. 1962. Action of actinomycin D on animal cells and viruses. Proc. Nat. Acad. Sci. U.S.A. **48**:1238–1245.
22. Riggs, A. D., G. Reiness, and G. Zubay. 1971. Purification and DNA-binding properties of the catabolite gene activator protein. Proc. Nat. Acad. Sci. U.S.A. **68**:1222–1225.
23. Silverstone, A. E., R. R. Arditti, and B. Magasanik. 1970. Catabolite-insensitive revertants of *lac* promoter mutants. Proc. Nat. Acad. Sci. U.S.A. **66**:773–779.
24. Sippel, A., and G. Hartmann. 1968. Mode of action of rifamycin on the RNA polymerase reaction. Biochim. Biophys. Acta **157**:218–219.
25. Sirbasku, D. A., and J. M. Buchanan. 1970. Patterns of ribonucleic acid synthesis in T5-infected *Escherichia coli*. II. Separation of high molecular weight ribonucleic acid species by disc electrophoresis on acrylamide gel columns. J. Biol. Chem. **245**:2679–2692.
26. Sirbasku, D. A., and J. M. Buchanan. 1971. Patterns of ribonucleic acid synthesis in T5-infected *Escherichia coli*. V. Formation of stable, discrete, degradation products during turnover of phage-specific ribonucleic acid. J. Biol. Chem. **246**:1665–1676.
27. Travers, A. 1970. RNA polymerase and T4 development. Cold Spring Harbor Symp. Quant. Biol. **35**:241–251.
28. Walter, G., W. Seifert, and W. Zillig. 1968. Modified DNA-dependent RNA polymerase from *E. coli* infected with bacteriophage T4. Biochem. Biophys. Res. Commun. **30**:240–247.
29. Zillig, W., K. Zechel, D. Rabussay, M. Schachner, V. S. Sethi, P. Palm, A. Heil, and W. Seifert. 1970. On the role of different subunits of DNA-dependent RNA polymerase from *E. coli* in the transcription process. Cold Spring Harbor Symp. Quant. Biol. **35**:47–58.
30. Zubay, G., D. Schwartz, and J. Beckwith. 1970. Mechanism of activation of catabolite-sensitive genes: a positive control system. Proc. Nat. Acad. Sci. U.S.A. **66**:104–110.