

# Replication of Mycoplasma virus MVL51

## IV. Inhibition of Viral Synthesis by Rifampin

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The effect of rifampin on the replication of MVL51, a bullet-shaped mycoplasma virus with single-stranded circular DNA of molecular weight  $2 \times 10^6$ , has been examined in a rifampin-resistant host cell. Rifampin does not block the early steps in MVL51 infection but does decrease the total amount of parental viral DNA taken up. The single-stranded parental viral DNA that enters the cell is found in membrane-associated, double-stranded DNA replicative forms I and II. Rifampin had no significant effect on the synthesis of progeny viral DNA RFI and RFII early in infection and SSI (single-stranded progeny viral chromosomes) later in infection. The rifampin block in virus synthesis was found to be in the step converting SSI into assembled virions. Rifampin was shown to affect the synthesis of virus-specific RNA, which suggests that viral transcription is necessary for virion assembly.

MVL51 is a group L1 mycoplasma virus, which are bullet-shaped particles (about 15 by 80 nm) having helical capsid symmetry and containing single-stranded circular DNA of molecular weight  $2 \times 10^6$  (8, 10). Viral infection is nonlytic, and infected cells continue to grow and extrude progeny viruses (9). Replication of MVL51 has been shown to involve three viral DNA intermediates: RFI, covalently closed circular double-stranded DNA; RFII, a nicked form of RFI; and SSI, a circular, single-stranded progeny viral DNA. RFI and RFII are precursors for the synthesis of SSI (6).

The antibiotic rifampin specifically inhibits initiation of transcription by RNA polymerase. In this paper, we report the effect of rifampin on the synthesis of MVL51 in a rifampin-resistant host. These data show that virus production is inhibited at concentrations of the antibiotic that have no effect on cell growth. The antibiotic does not inhibit synthesis of virus-specific DNAs but inhibits synthesis of some virus-specific RNA required for maturation and/or release.

### MATERIALS AND METHODS

**Cells, viruses, medium, and buffer.** *Acholeplasma laidlawii* JA1 was used for virus growth and as the indicator host in these studies. The virus used was MVL51, a group L1 mycoplasma virus described by Liss and Maniloff (9). Cells and viruses were cultivated in tryptose broth or on tryptose agar plates as described previously (9).

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The Tris-EDTA-NaCl buffer (pH 8.0) used was 0.01 M Tris, 0.001 M EDTA, 0.1 M NaCl, and Tris-EDTA-NaCl-NaCN buffer was the same buffer containing 10 mM NaCN. The high-salt buffer was Tris-EDTA-NaCl containing 1 M NaCl.

**Antibiotics.** Rifampin and chloramphenicol were obtained from Sigma Chemical Co. (St. Louis, Mo.). Rifampin solutions were made up in Tris-EDTA-NaCl buffer 1 day before use and stored at 4 C in the dark.

**Preparation of labeled virus.** Radioactively labeled MVL51 and viral DNA were prepared as described previously (6).

**Analysis of whole cells.** For examination of the effects of antibiotics on the incorporation of radioisotopically labeled compounds in whole cells, log-phase cultures of JA1 cells were treated with either rifampin or chloramphenicol. After different times 5-ml aliquots were removed, centrifuged, and suspended in 5 ml of Eagle basal medium containing the antibiotic and either 10  $\mu$ Ci of [<sup>3</sup>H]uridine or 20  $\mu$ Ci of L-[<sup>3</sup>H]amino acid mixture per ml (New England Nuclear Corp., Boston, Mass.). The rate of incorporation of labeled precursors into cellular macromolecules has been found to be better in this labeling medium than in the tryptose growth medium (13). After 3 min of labeling, cells were precipitated by adding 5 ml of ice-cold 10% trichloroacetic acid. The precipitate was collected on membrane filters (0.45- $\mu$ m pore size; Millipore Corp.), washed, and counted for radioactivity.

**Preparation of virus-infected cell lysates.** A log-phase culture of *A. laidlawii* JA1 was infected with either <sup>32</sup>P-labeled or unlabeled virus. To remove unadsorbed virus, after 5 min cells were spun down at 12,000  $\times g$  for 10 min, and the pellet was washed with Tris-EDTA-NaCl and suspended in prewarmed tryptose broth. At different times after infection,

antibiotic was added to the infected culture, and, after a specified interval, the cells were labeled for varying periods with [<sup>3</sup>H]deoxythymidine. Labeling was terminated by adding an equal volume of ice-cold Tris-EDTA-NaCl-NaCN buffer and centrifuging at  $12,000 \times g$  for 10 min at 5 C. The cell pellet was washed and lysed by 0.4% (final concentration) Sarkosyl NL97 as described previously (6). For isolating membrane-associated viral DNA, the infected cell suspension was gently lysed by freezing in liquid nitrogen and thawing (J. Das and J. Maniloff, Proc. Natl. Acad. Sci. U.S.A., in press).

**Sucrose gradient centrifugation.** One to two milliliters of an infected cell lysate was layered on top of 38 ml of a 5 to 20% (wt/vol) sucrose gradient in Tris-EDTA-1 M NaCl buffer. For isolating membrane-associated viral DNA, a 5 to 20% high-salt sucrose gradient was formed over a CsCl shelf. The gradients were centrifuged and fractionated as described previously (Das and Maniloff, in press).

**Radioactive assay.** Gradient fractions were precipitated by adding an equal volume of ice-cold 10% trichloroacetic acid, using bovine serum albumin as carrier. The precipitated samples were filtered, washed, dried, and assayed for radioactivity as previously described (6).

**RNA-DNA hybridization.** Pulse-labeled RNA was isolated from uninfected, infected, and infected rifampicin-treated JA1 cells by the method described by Mitra (11). For the infected cell experiment, a log-phase JA1 culture was infected with MVL51 at a multiplicity of infection of about 10. Thirty minutes after infection, rifampin (100  $\mu\text{g/ml}$ ) was added to part of the infected culture. Twenty-five minutes later, cells were labeled for 5 min with 40  $\mu\text{Ci}$  of [<sup>3</sup>H]uridine per ml. At the end of the labeling period, cells were rapidly chilled, centrifuged, washed with saline phosphate, and, finally, suspended in 0.05 M sodium acetate (pH 5.0). Sodium dodecyl sulfate (0.5% final concentration) and polyvinyl sulfate (10  $\mu\text{g/ml}$  final concentration) were then added. After 5 min at 4 C, the cell suspensions were extracted twice with phenol saturated with 0.15 M NaCl, 0.015 M sodium citrate (SSC), pH 7.0. The phenol phase was reextracted with 0.05 M sodium acetate (pH 5.0). The aqueous layers were pooled and precipitated with 3 volumes of ethanol at  $-20\text{ C}$  for 24 h. The nucleic acid precipitate was collected by centrifugation at  $4,000 \times g$  for 10 min and resuspended in SSC.

MVL51 replicative form (RF) DNA was isolated from sucrose gradients as previously described (6), dialyzed against SSC, and denatured by heating at  $100\text{ C}$  for 10 min and chilling rapidly.

The reaction mixture for hybridization contained 0.5 M KCl, 0.01 M Tris-hydrochloride (pH 7.5), pulse-labeled RNA, and denatured RF DNA. The reaction mixture was incubated for 30 min at 67 C and then cooled to room temperature. After 20 min at room temperature, pancreatic RNase (10  $\mu\text{g/ml}$ ) was added, and after 1 min the mixture was diluted with 5 volumes of KCl-Tris-hydrochloride buffer, filtered through a 0.45- $\mu\text{m}$  membrane filter (Millipore Corp.), washed twice with the same buffer, dried, and counted.

**Assay of RNA polymerase.** Uninfected and infected cells, after 60 min of infection in tryptone broth, were centrifuged at  $7,500 \times g$  for 10 min and suspended in 1 ml of buffer A (0.05 M Tris, 0.05 M EDTA, 0.01 M  $\text{MgCl}_2$ , 0.01 M  $\beta$ -mercaptoethanol, pH 8.0) (3). The cells were disrupted by sonication and centrifuged at  $12,000 \times g$  for 10 min. The supernatant was decanted, 0.2 volume of 1% protamine sulfate was added, and the mixture was centrifuged at  $12,000 \times g$  for 10 min. The precipitate was resuspended in 1 ml of buffer A. For the RNA polymerase assay, 100  $\mu\text{l}$  of the resuspended precipitate was used without further purification. The enzyme was assayed by the method of Berg et al. (2), using [<sup>3</sup>H]uridine as labeled nucleotide and highly polymerized calf thymus DNA (150  $\mu\text{g/ml}$ ) as template. Potassium phosphate (20 mM) was added to the reaction mixture to inhibit polynucleotide phosphorylase. The enzyme fractions were preincubated for 5 min at room temperature before adding the DNA. The enzyme activity was measured after 10 min of incubation at room temperature and corrected for endogenous DNA by measuring incorporation of [<sup>3</sup>H]uridine in the absence of added DNA.

## RESULTS

**Growth of infected and uninfected cells in the presence of rifampin.** During studies of the effect of rifampin on the replication of mycoplasma virus MVL51 (to be described below), it was observed that the *A. laidlawii* JA1 indicator host, which was used in these and all previous virus studies in this laboratory, was rifampin resistant. The effect of rifampin on the growth of uninfected and MVL51-infected *A. laidlawii* JA1 cells is shown in Fig. 1. Growth of uninfected cells (Fig. 1a) was not affected by low antibiotic concentrations (up to 50  $\mu\text{g/ml}$ ). At 100  $\mu\text{g/ml}$ , cell growth continued for 3 h; similar results were found at 200  $\mu\text{g/ml}$ . Growth of MVL51-infected cells in the presence of 100  $\mu\text{g}$  of rifampin per ml (Fig. 1b) was similar to that of infected untreated cells.

**Effect of rifampin on cellular RNA and protein synthesis.** Incorporation of [<sup>3</sup>H]uridine in cells treated with 100  $\mu\text{g}$  of rifampin per ml was not affected for 2 to 3 h after the addition of the drug, after which the incorporation was reduced (Fig. 2b). For comparison, in cells treated with 100  $\mu\text{g}$  of chloramphenicol per ml, uridine incorporation continued at the untreated control cell rate for 1 h and then stopped.

Protein synthesis (Fig. 2a), as measured by incorporation of labeled amino acids, was barely affected by 100  $\mu\text{g}$  of rifampicin per ml, whereas it was completely inhibited by 100  $\mu\text{g}$  of chloramphenicol per ml.

**Effect of rifampin on cellular RNA polymerase.** In vitro assays of the RNA polymerase activity in extracts from infected and unin-

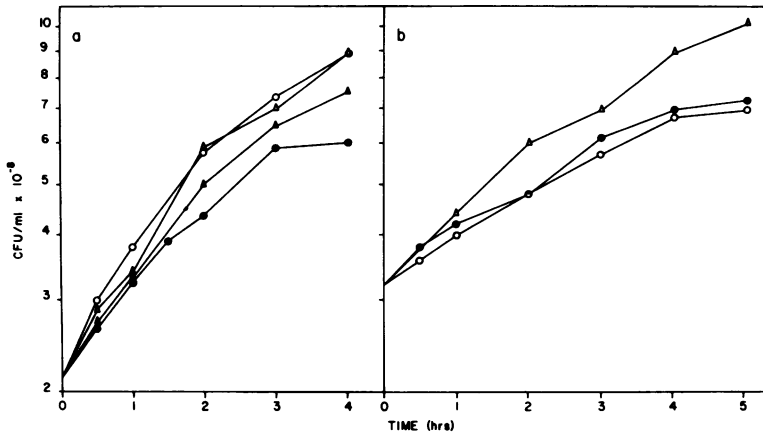


FIG. 1. (a) Growth of JA1 cells in the presence of rifampin. The drug concentrations were: (○) control; (△) 20 µg/ml; (▲) 50 µg/ml; and (●) 100 µg/ml. (b) Growth of MVL51-infected JA1 cells in the presence of 100 µg of rifampin per ml. Symbols: (△) uninfected and (○) infected cells without rifampin; (●) infected cells plus 100 µg of rifampin per ml.

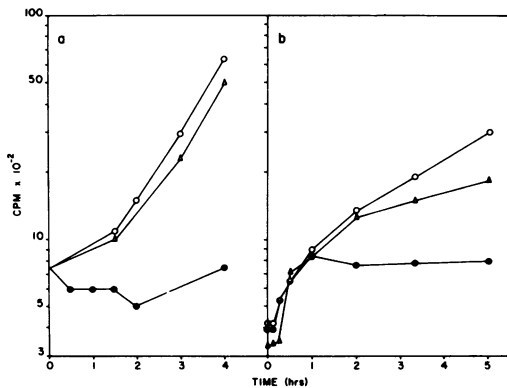


FIG. 2. Incorporation of (a) [<sup>3</sup>H]amino acids and (b) [<sup>3</sup>H]uridine into trichloroacetic acid-insoluble material after addition of (△) 100 µg of rifampin per ml, (●) 100 µg of chloramphenicol per ml, or (○) control.

infected *A. laidlawii* JA1 cells were performed in the presence of different concentrations of rifampin to examine the rifampin sensitivity of the RNA polymerase of these cells. The results (Fig. 3) show that the enzyme from both MVL51-infected and uninfected cells was resistant to rifampin. Even 100 µg of the antibiotic per ml only inhibited about 50% of the in vitro RNA polymerase activity.

**Viral growth in the presence of rifampin.** The effect of rifampin on MVL51 viral growth was examined. At various times after the start of infection, a sample of an infected cell culture was removed and treated with 100 µg of the antibiotic per ml. One hour after the addition of the drug, PFU were assayed. The untreated culture showed the characteristic growth curve

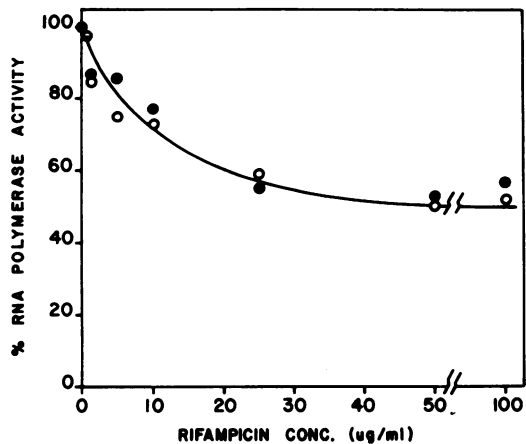


FIG. 3. RNA polymerase activity in extracts from infected and uninfected JA1 cells in the presence of rifampin. The activity is expressed as percentage of activity in the absence of the drug. Symbols: (○) uninfected cells; (●) cells infected with MVL51.

for this nonlytic virus (Fig. 4). Rifampin treatment inhibited the growth of the virus, but the extent of inhibition depended upon when during infection the antibiotic was added (Fig. 4a). The amount of inhibition increased with increasing infection times. For comparison, a parallel experiment was performed with 100 µg of chloramphenicol per ml. Chloramphenicol completely inhibited virus production independent of the time during infection of addition of the drug (Fig. 4b).

The effect of 2-h treatments of different rifampin concentrations is shown in Fig. 5. Rifampin concentrations, which had been found

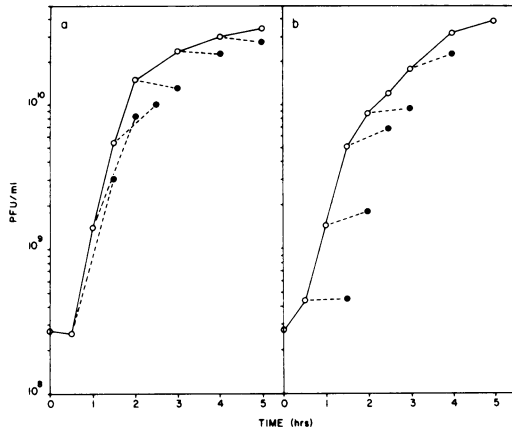


FIG. 4. Effect of rifampin and chloramphenicol on virus growth. A log-phase culture of JA1 cells was infected with MVL51 at a multiplicity of infection of 3. After 5 min, cells were centrifuged to remove unadsorbed viruses and infected cells were resuspended in tryptose broth. At different times after infection aliquots were removed and virus assays were performed, before and after 1-h treatments with either 100  $\mu\text{g}$  of (a) rifampicin or (b) chloramphenicol per ml. Symbols: (O) control; (●) virus titer after 1-h drug treatment.

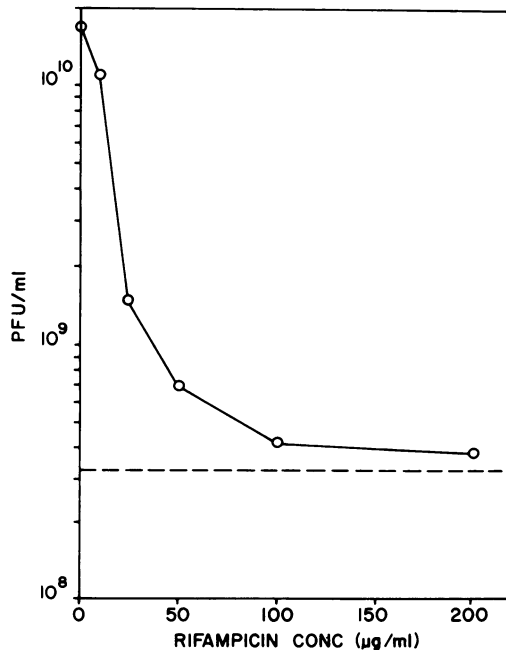


FIG. 5. Effect of rifampin concentration on virus yield. The protocol was as in the legend to Fig. 4, except that 5 min after infection the cells were transferred to medium containing different concentrations of rifampin. PFU were assayed 2 h after the transfer. The broken line indicates virus titer at the time of rifampin addition.

to have little or no effect on growth of uninfected (Fig. 1a) and infected cells (Fig. 1b), were found to inhibit viral production by 90% or more.

**Reversibility of rifampin effect.** To examine whether the effect of rifampin on virus growth could be reversed, 100  $\mu\text{g}$  of rifampin per ml was added to infected cells 100 min after infection and the culture was incubated for 1 h. At the end of this period the cells were removed from the antibiotic-containing medium, and the infected cell pellet was washed and resuspended in prewarmed tryptose broth. PFU were assayed before and after the removal of the antibiotic. PFU production was almost completely inhibited by 1 h of drug treatment at 100 min after infection. After the removal of the antibiotic, there was a lag of about 30 min, and then virus production began at about the same rate as untreated cells (Fig. 6).

**Effect of rifampin on virus-specific RNA synthesis.** The data presented above show that rifampin inhibits production of virus, although the RNA polymerases from infected and unin-

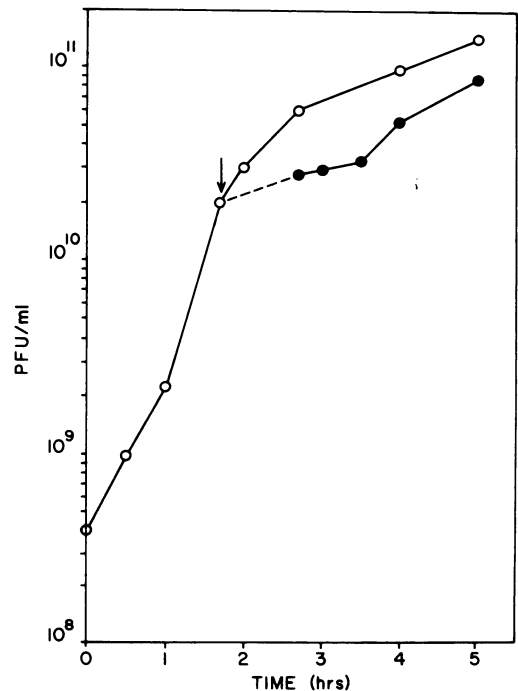


FIG. 6. Reversibility of the effect of rifampin on virus synthesis. Cells were infected as in the legend to Fig. 4. At 100 min after infection (arrow), an aliquot of infected cells was treated with 100  $\mu\text{g}$  of rifampin per ml for 1 h. The cells were then centrifuged, washed, and resuspended in fresh tryptose broth. Symbols: (O) control; (●) treated infected cells after rifampin removal.

ected cells are resistant to the drug. This effect could be due to rifampin only inhibiting the synthesis of virus-specific RNA. To test this possibility, pulse-labeled RNA was isolated from uninfected, MVL51-infected, and infected and rifampin-treated JA1 cells and hybridized with MVL51 RF DNA. The results are shown in Table 1. The amount of RNA hybridized was maximum when RNA from infected cells was used. This amount was reduced 2.5-fold when infected cells were treated with 100  $\mu\text{g}$  of rifampin per ml for 30 min. Hence, rifampin inhibits the synthesis of virus-specific RNA. The activity bound in the filters when RNA from uninfected cells was used may be due to cellular DNA contamination in the RF preparation.

**Effect of rifampin on parental RF formation.** To examine the effect of rifampin on the early steps in viral replication, JA1 cells were either pretreated with the antibiotic before infection or the antibiotic was added along with the virus. Infected cells were then analyzed for the amount of parental viral DNA in subcellular fast (membrane-associated)- and free-sedimenting fractions (Table 2). The total amount of parental viral DNA recovered in the cells (i.e., in the fast- and free-sedimenting fractions) was reduced when rifampin was added at the start of infection and decreased further by 30-min pretreatment of the cells by rifampin before infection. Longer rifampin pretreatment (up to 60 min) caused no further decrease in recovery of intracellular parental viral DNA (data not shown).

The distribution of parental label shows a marked decrease in the amount of free-sedimenting parental viral DNA relative to fast-sedimenting DNA in rifampin-treated infected cells compared to untreated infected cells. Hence, rifampin treatment decreases the amount of uptake of parental viral DNA and shifts the intracellular distribution of the viral

DNA, which is incorporated so that it is almost totally recovered in a membrane-associated form. The result of these two effects is that only 60% of the parental viral DNA that can be recovered as fast-sedimenting material in untreated cells is found as fast-sedimenting material in rifampin-treated cells. The free-sedimenting parental viral DNA in rifampin-treated cells is reduced to 7% of that found in untreated cells.

To examine the conversion of parental DNA to RF forms, cells were either untreated or treated for 30 min with 100  $\mu\text{g}$  of antibiotic per ml before infection with labeled viruses. Ten minutes after infection, the cells were washed, lysed by Sarkosyl NL97, and sedimented through a 5 to 20% sucrose gradient containing 1 M NaCl, as described in Materials and Methods. The single-stranded DNA of MLV51 was found in double-stranded replicative forms RFI and RFII (Fig. 7a). This conversion was not affected by pretreating cells for 30 min with 100  $\mu\text{g}$  of rifampin (Fig. 7b) or 100  $\mu\text{g}$  of chloramphenicol (Fig. 7c) per ml, indicating that preexisting cell enzyme(s) and cell-specific transcription can perform the initial steps of MVL51 DNA replication.

**Effect of rifampin on synthesis of progeny viral DNA.** To examine the effect of rifampin on RF replication and single-stranded progeny viral DNA synthesis, infected cells were treated for 30 min with 100  $\mu\text{g}$  of rifampin per ml at 0 and 90 min after infection. During the last 10 min of antibiotic treatment at each time, [ $^3\text{H}$ ]deoxythymidine was added to the culture to label the nascent DNA. A parallel infected culture was not rifampin treated.

It has been shown (6) that, during the first 30 to 40 min of MVL51 infection, RF replication predominates, after which there is a shift to the synthesis of SSI. The synthesis of progeny viral RF molecules in untreated cells and cells treated with rifampin during the first 30 min of infection was similar (Fig. 8a). Infected cells treated with rifampin during 90 to 120 min after infection made progeny SSI, but no nascent viral DNA could be found in the gradient fraction corresponding to completed virus particles (Fig. 8b). Lysates of untreated infected cells late in infection show this virus peak, since the lysis procedure used does not disrupt virus particles (6). The peak must represent viruses in the process of being extruded.

The amount of nascent virus-specific DNA found in the lysates of rifampin-treated cells was greater than in untreated cells (Fig. 8b). This difference could account for the amount of cold trichloroacetic acid-precipitable radioactivity in the cell-free supernatant of the untreated

TABLE 1. Hybridization of  $^3\text{H}$ -labeled RNA with MVL51 RF DNA

Source of RNA	Input (counts/min)	Sp act (counts/min per $A_{260}^a$ )	$^3\text{H}$ bound to membrane filter containing RF DNA (counts/min)
Uninfected cells	$1.40 \times 10^4$	$8.2 \times 10^3$	90
Infected cells	$1.37 \times 10^4$	$7.7 \times 10^3$	446
Infected cells + 100 $\mu\text{g}$ of rifampin per ml	$1.43 \times 10^4$	$8.9 \times 10^3$	173

<sup>a</sup>  $A_{260}$ , Absorbancy units at 260 nm.

TABLE 2. Effect of rifampin pretreatment on penetration and membrane association of viral DNA

Treatment <sup>a</sup>	Total amt of parental viral DNA in cells		Distribution of parental label			
	Counts/min	% Relative to untreated cells	Fast-sedimenting complex		Free-sedimenting material	
			Counts/min	% of total counts/min	Counts/min	% of total counts/min
Untreated	1,167	100	836	72	331	28
Rifampin (100 $\mu$ g/ml) added at start of infection	831	71	735	88	96	12
Infection started after 30-min rifampin (100 $\mu$ g/ml) pre-treatment	522	45	500	96	22	4
Infection started after 30-min rifampin (200 $\mu$ g/ml) pre-treatment	498	43	478	96	20	4

<sup>a</sup> Infection was with <sup>32</sup>P-labeled MVL51 at a multiplicity of infection of 10. Fast- and free-sedimenting material were analyzed, as described in Materials and Methods, 10 min after the start of infection.

infected cells. Hence, the difference in amounts of progeny viral DNA between untreated and treated cells in Fig. 8b is due to the release of nascent DNA in progeny viruses from the untreated cells.

To examine whether the single-stranded progeny viral DNA accumulated during rifampin treatment could be assembled into progeny particles upon removal of the drug, part of the rifampin-treated sample (shown in Fig. 8b) was centrifuged and the cells were resuspended in prewarmed tryptose broth to remove the rifampin. Forty minutes after resuspension, a cell lysate was prepared and sedimented through a high-salt sucrose gradient. It was found (Fig. 8c) that some of the virus-specific DNA synthesized during rifampin treatment could now be recovered at the virus particle position. The total amount of radioactivity in intracellular viral DNA decreased after rifampin removal (cf. Fig. 8b and c) but was recovered as cold trichloroacetic acid-insoluble material in the cell-free supernatant, indicating the release of progeny virus.

## DISCUSSION

The indicator host used in these studies, *A. laidlawii* JA1, is rifampin resistant, as shown by the inability of rifampin to inhibit cell growth (Fig. 1a) and RNA and protein synthesis (Fig. 2). In considerations of whether the rifampin resistance could be due to a permeability barrier, it should be noted that *A. laidlawii*, like other mycoplasmas, does not have a

cell wall and is bounded by a single 7-nm lipoprotein cell membrane (4). The observation that virus (but not cell) growth is inhibited by rifampin treatment (Fig. 1b and 4) means that the antibiotic must be getting into the cell. The resistance of cellular RNA polymerase activity to rifampin, in an in vitro assay (Fig. 3), confirms the whole-cell data.

The results presented here show that rifampin does not block the early steps in MVL51 infection but does decrease the total amount of parental viral DNA taken up by the indicator cells. The single-stranded parental viral DNA that enters the cell is found in membrane-associated, double-stranded DNA RFI and RFII (Fig. 7; Table 2). Rifampin had no significant effect on the synthesis of progeny viral DNAs; early in infection this synthesis was mainly in RF molecules and later in infection in SSI DNA (Fig. 8a and b). The rifampin block in virus synthesis was seen to be in the step converting SSI (progeny viral chromosomes) into assembled virions. Removal of rifampin allowed these progeny SSI molecules to be packaged and released (Fig. 8c).

Parenthetically, it was noted that late in infection rifampin treatment led to a larger accumulation of RFII molecules, relative to RFI and SSI, than would be expected at this infection time (Fig. 8b). This suggests the possible existence of a reusable protein (similar to the gene 5 product of bacteriophage M13; 12) mediating the conversion of RFII to SSI. The rifampin block in virus assembly, causing the accumula-

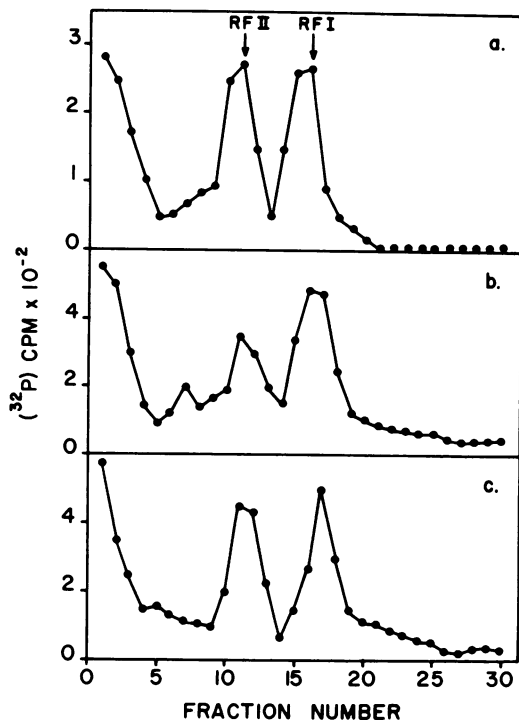


FIG. 7. Velocity sedimentation of infected cell lysates. JA1 cells were grown to  $5 \times 10^8$  cells/ml and treated with either 100  $\mu$ g of rifampin or 100  $\mu$ g of chloramphenicol per ml for 30 min. <sup>32</sup>P-labeled MVL51 was then added to a multiplicity of infection of 20. After 10 min at 37 C, unadsorbed viruses were removed by washing and the infected cell pellet was resuspended in ice-cold Tris-EDTA-NaCl buffer. Cells were lysed by 0.4% Sarkosyl NL97, layered on 5 to 20% sucrose gradients containing 1 M NaCl, and centrifuged at 24,000 rpm for 16 h in an SW27 rotor. The direction of sedimentation was from left to right. (a) Untreated; (b) rifampin treated; (c) chloramphenicol treated. The SSI position was determined from a parallel gradient of purified viral DNA, and the RFI and RFII positions were calculated from their sedimentation rates relative to SSI, as given in Das and Maniloff (6).

tion of SSI molecules, would exhaust the pool of such a protein, resulting in the buildup of RFII. Experiments to explicitly demonstrate and isolate this protein are in progress.

Rifampin was shown to affect the synthesis of some virus-specific RNA (Table 1). This observation, together with the data that the rifampin block in virus synthesis is in the conversion of progeny viral chromosomes into assembled particles, suggests that virus-specific transcription is necessary for this assembly step. The rifampin sensitivity of this viral transcription in rifampin-resistant cells could be due to several possibilities. First, the MVL51 mycoplas-

mavirus, like coliphage T7 (5), could code for its own RNA polymerase. In view of the limited coding capacity of MVL51 and the large size of such a polymerase, this possibility appears unlikely. Second, a viral gene product or a cellular virus-specific factor, similar to those reported for coliphages T4 (14) and  $\lambda$  (7), might modify

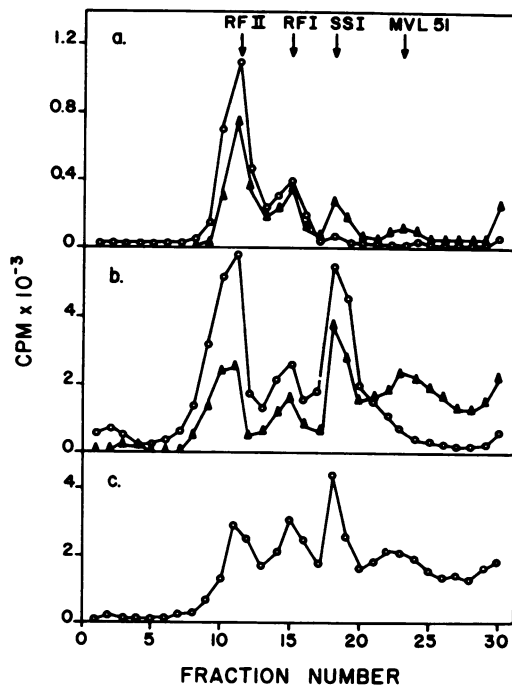


FIG. 8. Velocity sedimentation of infected cells treated with rifampin at different times during infection. JA1 cells were grown to  $5 \times 10^8$  cells/ml, and MVL51 was added to a multiplicity of infection about 10. After 5 min at 37 C, the cells were spun down and resuspended in prewarmed tryptose broth. At 0 and 90 min after resuspension, 15-ml aliquots were removed and 100  $\mu$ g of rifampicin per ml was added for 30 min. During the last 10 min at each time point, cells were labeled with 40  $\mu$ Ci of [<sup>3</sup>H]deoxythymidine per ml. Labeling was terminated by adding an equal volume of ice-cold Tris-EDTA-NaCl-NaCN buffer. The infected cells were washed, mixed with <sup>32</sup>P-labeled marker MVL51 DNA lysed by 0.4% Sarkosyl, and sedimented in 5 to 20% high-salt sucrose gradients for 16 h at 24,000 rpm using an SW27 rotor. The direction of sedimentation is from left to right. (a) Treated from 0 to 30 min with rifampin; (b) treated from 90 to 120 min with rifampin. Symbols: ( $\blacktriangle$ ) untreated and ( $\circ$ ) treated infected cells. (c) Part of the rifampin-treated sample in (b) was centrifuged at 12,000 rpm for 5 min at 4 C, washed, and resuspended in prewarmed tryptose broth. Forty minutes after resuspension, cell lysate was prepared and centrifuged as described above. The RFI and RFII positions, relative to the marker, were calculated as in the legend to Fig. 7.

the cells' RNA polymerase, making it rifampin sensitive. Finally, the viral transcription complex might be different from that of the cell, since it has been shown that RNA polymerase can bind at the promotor site in two configurations, one sensitive and one resistant to rifampin (1).

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#### LITERATURE CITED

1. Bautz, E. K. F. 1972. Regulation of RNA synthesis. *Prog. Nucleic Acid Res. Mol. Biol.* 12:129-160.
2. Berg, D., K. Barrett, and M. Chamberlin. 1971. Purification of two forms of *Escherichia coli* RNA polymerase and of sigma component, p. 506-519. In L. Grossman and K. Moldave (ed.), *Methods in enzymology*, vol. 21D. Academic Press Inc., New York.
3. Burtlag, D., R. Schekman, and A. Kornberg. 1971. A possible role for RNA polymerase in the initiation of M13 DNA synthesis. *Proc. Natl. Acad. Sci. U.S.A.* 68:2826-2829.
4. Carstensen, E. L., J. Maniloff, and C. W. Einolf, Jr. 1971. Electrical properties and ultrastructure of mycoplasma membranes. *Biophys. J.* 11:572-581.
5. Chamberlin, M., and J. Ring. 1973. Characterization of T7-specific ribonucleic acid polymerase. *J. Biol. Chem.* 248:2245-2250.
6. Das, J., and J. Maniloff. 1975. Replication of mycoplasma virus MVL51. I. Replicative intermediates. *Biochem. Biophys. Res. Commun.* 66:599-605.
7. Kornberg, A. 1974. DNA synthesis, p. 345. W. H. Freeman and Co., San Francisco.
8. Liss, A., and J. Maniloff. 1973. Characterization of Mycoplasma virus DNA. *Biochem. Biophys. Res. Commun.* 51:214-218.
9. Liss, A., and J. Maniloff. 1973. Infection of *Acholeplasma laidlawii* by MVL51 virus. *Virology* 55:118-126.
10. Maniloff, J., and A. Liss. 1974. Comparative structure, chemistry and evolution of mycoplasma viruses, p. 583-604. In E. Kurstak and K. Maramorosch (ed.), *Viruses, evolution and cancer*. Academic Press Inc., New York.
11. Mitra, S. 1972. Inhibition of M13 phage synthesis by rifampin in some rifampin resistant *Escherichia coli* mutants. *Virology* 50:422-430.
12. Pratt, D., P. Laws, and J. Griffith. 1974. Complex of bacteriophage M13 single stranded DNA and gene 5 protein. *J. Mol. Biol.* 82:425-439.
13. Quinlan, D. C., A. Liss, and J. Maniloff. 1972. Eagle's basal medium as a defined medium for mycoplasma studies. *Microbios* 6:179-185.
14. Stevens, A. 1972. New small polypeptides associated with DNA dependent RNA polymerase of *Escherichia coli* after infection with bacteriophage T4. *Proc. Natl. Acad. Sci. U.S.A.* 68:603-607.