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Inhibition by Lipiarmycin of Bacteriophage Growth in *Bacillus subtilis*

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We have used lipiarmycin, a specific inhibitor of initiation of transcription, to study the role of host RNA polymerase in the transcription programs of various phages of *Bacillus subtilis*. Unlike rifampin, lipiarmycin preferentially inhibits transcription dependent on the σ subunit of RNA polymerase because it inactivates holoenzyme at a much greater rate than it does core enzyme. With phage SP01, addition of lipiarmycin at a middle-to-late time of infection did not inhibit phage production even though phage production was sensitive to addition of rifampin at that time. This result is consistent with the notion that unmodified host RNA polymerase holoenzyme becomes dispensable after transcription of early classes of SP01 genes, even though host core enzyme is required for synthesis of all classes of phage RNA. SP01-modified forms of RNA polymerase, which lack σ subunit but contain phage-coded polypeptides and are able to transcribe middle and late genes, were resistant to lipiarmycin in vitro. For phage ϕ105, phage development was sensitive to both lipiarmycin and rifampin in wild-type cells and resistant to both drugs in resistant mutant cells, leading to the conclusion that the activity of host holoenzyme was required for phage RNA synthesis. Growth of phage PBS2, which was resistant to rifampin, was sensitive to the addition of lipiarmycin at early times of infection of a wild-type host strain. In a lipiarmycin-resistant mutant host, PBS2 growth was resistant to lipiarmycin. This result suggests that host holoenzyme plays a previously unanticipated role in transcription of PBS2 genes.

Lipiarmycin inhibits the growth of many gram-positive bacteria (2). In *Bacillus subtilis*, the drug acts by inhibiting initiation of RNA synthesis by DNA-dependent RNA polymerase (19, 20).

A number of lipiarmycin-resistant mutants of *B. subtilis* have been isolated in our laboratory. In most strains, the resistance mutation maps between loci for rifampin resistance and streptolydigin resistance (21), suggesting that the binding site for lipiarmycin involves the β or β' (or both) subunit of RNA polymerase (6, 7). These mapping data are consistent with our finding that in at least one case the lipiarmycin resistance property of mutant RNA polymerase was associated with the core enzyme fraction (21).

Unlike other RNA polymerase inhibitors, such as rifampin and streptolydigin, lipiarmycin inhibits transcription by RNA polymerase molecules that are in the holoenzyme form much more efficiently than it inhibits transcription by core polymerase. This suggests that the action of the drug is primarily on sigma-dependent transcription (20, 21). The mechanisms of action of lipiarmycin and rifampin can be further distinguished by the fact that RNA polymerase-DNA complexes (so-called rapid-starting complexes) can be inactivated by rifampin but not by lipiarmycin (20). The differences between lipiarmycin and other inhibitors, in particular the preferential inhibition of holoenzyme by lipiarmycin, suggested to us that lipiarmycin might be useful in analyzing transcription during phage infection of *B. subtilis*, since the effect of the drug on phage growth could provide information about the role of host sigma factor during infection. We began these studies by analyzing the effect of lipiarmycin on the growth of phage SP01, a phage that induces modifications of host RNA polymerase during infection (9), phage ϕ105, for which the enzymes responsible for phage transcription have not been described, and phage PBS2, which has been thought to cause replacement of host RNA polymerase by phage-coded enzymes (15, 17).

MATERIALS AND METHODS

Bacterial and phage strains. Bacterial strains are listed, with their relevant properties, in Table 1. Strain LS121 was a spontaneous mutant of 3610 able

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TABLE 1. *Bacterial strains*

Strain	Genotype[a]	Source or reference
3610	Wild type	Laboratory collection
LS2	*rfm-11*	Laboratory collection; 22
LS121	*lpm-121*	Spontaneous mutant of 3610
LS1213	*lpm-121 rfm-12*	Spontaneous mutant of LS121
168 (ϕ105 wild type)	*trpC2*	L. Rutberg
JAS5	*purB6 leuA8 metB5 car-41*	J. A. Shapiro
LS105	*purB6 leuA8 metB5 car-41 lpm-105*	Spontaneous mutant of JAS5; 21
DR10	*std-10*	Spontaneous mutant of W168; D. M. Rothstein, Ph.D. thesis, Tufts University, 1977

[a] Symbols used are those of Young and Wilson (26). The genotype *lpm* denotes resistance to lipiarmycin.

to grow on rich (DSM) plates containing 20 μg of lipiarmycin per ml. Strain LS1213 was a spontaneous derivative of LS121 able to grow on DSM plates containing 5 μg of rifampin per ml. Phage strains SP01 and PBS2 were obtained from J. Pero. ϕ105 wild-type was obtained from L. Rutberg; ϕ105c30, a clear-plaque mutant (18), was provided by J. A. Shapiro.

Media. DSM, L, and TBAB media and nutrient top agar have been described (22). Modified 121A medium contained basal 121 medium (23) plus 0.8% glucose, 2.5 × 10^{-3} M K_2HPO_4, 0.05% casein hydrolysate, and 40 μg each of $FeCl_3$ and trisodium citrate per ml. 121 plates and 121 top agar were identical and contained, per liter, 900 ml of water, 100 ml of 10-times-concentrated 121 basal medium, and 10 g of agar. After autoclaving, glucose was added to 0.8% final concentration, casein hydrolysate to 0.01%, and K_2HPO_4 to 10^{-3} M. PBS1 buffer contained, per liter, 1 g of yeast extract, 4 g of NaCl, 5 g of K_2HPO_4, and 3 g of $NaHPO_4$; 3 ml of 2% $MgSO_4 \cdot 7H_2O$, 0.4 ml of 0.1 M $CaCl_2$, and 0.02 ml of 0.1 M $FeCl_3 \cdot 6H_2O$ were added after autoclaving.

Antibiotics. Rifampin was purchased from Calbiochem. The drug was dissolved in 50% ethanol (at 1 mg/ml), sterilized by filtration, and stored at −20°C for up to 1 week. Lipiarmycin, the gift of G. C. Lancini, Gruppo Lepetit, Milan, was freshly prepared for each use by dissolving in 95% ethanol (at 1 to 2.5 mg/ml). Mitomycin C (Sigma Chemical Co.) was dissolved in sterile water (at 1 mg/ml) and stored in the dark at 4°C. Streptolydigin, the gift of G. B. Whitfield, Jr., The Upjohn Co., was dissolved in 95% ethanol. For control experiments, an amount of ethanol was added equivalent to that added with drug.

Phage lysates and plating conditions. Conditions for growing and assaying phage ϕ105 have been previously described (11).

To prepare SP01 lysates, *B. subtilis* strain 3610 was grown in L medium to 5 × 10^8 cells/ml (150 Klett units) and infected at a multiplicity of 0.05. Infected cells were incubated with shaking at 37°C until lysis (4 to 6 h) and stored overnight at 4°C. Cell debris was removed by centrifugation, and lysates were stored at 4°C over a crystal of thymol. Phage were diluted in L medium and plated on fresh L plates at 37°C in nutrient top agar overlay, using logarithmic-phase cells of strain 3610 as indicator.

PBS2 was prepared from plate lysates. Phage were diluted in PBS1 buffer and plated as follows: 0.1 ml containing about 10^4 PFU and 0.1 ml of strain 3610 grown in modified 121A medium to late logarithmic phase (the period of active motility) were mixed in a tube containing 1.4 ml of PBS1 buffer. The tube was incubated at room temperature for 3 to 5 min without shaking to allow phage adsorption. Melted 121A top agar (1.5 ml) was then added, and the contents were poured onto 121 plates. Plates were incubated overnight at 30°C. The next day, 5 ml of PBS1 buffer was pipetted onto each plate, and the plates were incubated at 4°C for 30 min. Top agar overlays were then scraped off, using sterile glass spreaders, and centrifuged to remove lumps of agar and cell debris. Lysates were sterilized by passage through a membrane filter (0.45-μm pore size; Millipore Corp.) and stored at 4°C. Typical titers of plate lysates were 10^9 to 10^{10} PFU per ml.

Determination of intracellular phage. Infected cells were killed and disrupted by adding sodium azide (to 0.1%) and lysozyme (to 300 μg/ml) to samples of the infected culture and incubating the cells at 0°C for 60 min. Intracellular phage titers were then determined by plating samples according to the methods described above.

Cell growth. All liquid cultures were inoculated from TBAB plates that had been incubated overnight at 30°C. Turbidity changes were monitored with a Klett-Summerson colorimeter (green filter).

RNA polymerase assays. RNA polymerase activity was assayed as described by Losick and Sonenshein (10) except that the specific activity of [^{14}C]ATP was 4 to 6 mCi/mmol. Enzymes B and C, purified from SP01-infected cells of strain 3610 by phosphocellulose chromatography, purified preparations of 3610 holoenzyme and 3610 core polymerase, and SP01 DNA were kindly provided by J. Pero and G. Lee. Poly(deoxyadenylic-deoxythymidylic)acid [poly(dA-dT)] was purchased from Miles Laboratories.

RESULTS AND DISCUSSION

SP01. After infection of *B. subtilis* by phage SP01, the synthesis of at least six classes of SP01 RNA (e, em, m, m_1l, m_2l, and l) can be distinguished (5). Only the two early classes (e and em) are made in the presence of chloramphenicol (5). The fact that synthesis of all classes of SP01 transcripts is rifampin sensitive in a wild-type (Rfm^s) host and rifampin resistant in an Rfm^r mutant host (6) indicates that a component of host polymerase is used throughout infection. Current evidence suggests that early RNA synthesis is directed by host RNA polymerase containing sigma factor, whereas middle and late phage transcription is mediated by modified forms of host polymerase in which sigma is replaced by phage-coded proteins (9, 12, 14, 25).

Since lipiarmycin differs from rifampin in its mechanism and specificity of action, we measured the effect of lipiarmycin on the growth of SP01 in wild-type and lipiarmycin-resistant hosts. Growth of SP01 was sensitive to lipiarmycin in the Lpm^s host JAS5, but was resistant to lipiarmycin in the Lpm^r strain LS105 (Table 2). (Strain LS105 has been shown to have a core mutation responsible for the lipiarmycin resistance of its RNA polymerase [21]). This result is consistent with the notion that no lipiarmycin-sensitive RNA polymerase independent of the host enzyme is required for successful infection. To find out when during the infection process lipiarmycin was inhibitory, infected cells of the Lpm^s strain 3610 were treated at various times after infection and tested for their ability to produce a phage burst (Fig. 1). Phage production was very sensitive to lipiarmycin when the drug was added at any time up to 15 min after infection, but resistant thereafter. By contrast, sensitivity to rifampin continued for at least 5 min after growth began to be lipiarmycin resistant. The fraction of the eclipse period during which phage growth was rifampin sensitive was similar to that reported by other laboratories (see reference 5), although the actual eclipse and latent periods were longer than those described by others. The fraction of the eclipse period during which phage growth was lipiarmycin sensitive corresponds roughly to the time during which early classes of RNA are known to be synthesized. This suggested that enzymes responsible for middle and late transcription are less sensitive to lipiarmycin than is the unmodified host RNA polymerase; we were led to study the properties of these enzymes in vitro.

Middle RNA synthesis requires the function of phage gene 28; late transcription requires the functions of phage genes 33 and 34 (4). All of these gene products (gp) bind to RNA polymerase (for a review, see reference 9). Two enzymes, B and C, purified from SP01-infected cells by Pero and co-workers, were shown to mediate middle and late transcription, respectively (24). Enzyme B contains host core subunits plus gp28, and enzyme C contains the core subunits plus gp33 and gp34; neither enzyme contains host sigma subunit (12). We tested the effect of lipiarmycin on the in vitro transcription of SP01 DNA by the B and C enzymes from infected cells and by holoenzyme from uninfected cells. The phage-modified enzymes were much more resistant than holoenzyme and at least as resistant as core enzyme to lipiarmycin at 1 μg/ml, although all enzymes tested were sensitive to rifampin (Table 3). At higher lipiarmycin concentrations, enzyme B proved to be more sensitive than enzyme C (Fig. 2). When assayed with poly(dA-dT) as template, the RNA polymerase species showed the same hierarchy of lipiarmycin sensitivity (data not shown). Our results support the view that the sensitivity of SP01 infection to lipiarmycin is due to inhibition of early RNA synthesis by unmodified host holoenzyme. Moreover, one can conclude that there is a period of RNA synthesis starting at a middle-to-late time of infection that is independent of unmodified holoenzyme and, in particular, of the sigma subunit.

φ105. Previous information about φ105 transcriptional control indicates only that protein synthesis is required for the transition from early to late RNA synthesis (15). To investigate the role of host RNA polymerase in early and late transcription, we tested the effect of rifampin on

TABLE 2. *Burst sizes of SP01 and φ105 with and without drug treatment in various host strains*[a]

Phage	Host strain	Drug phenotype	Burst size		
			No drug	+Lipiarmycin[b]	+Rifampin[c]
SP01	3610	Wild type	50	0.04	0.03
	JAS5	Wild type	24	0.1	
	LS105	Lpm^r	73	46	
φ105 wild type[d]	168(φ105 wild type)	Wild type	22		0.1
φ105*c30*	3610	Wild type	100	0.4	0.1
	LS2	Rfm^r	200		280
	JAS5	Wild type	60	0.4	
	LS105	Lpm^r	112	190	

[a] Burst sizes were measured as PFU at 90 min divided by the number of infective centers (PFU at 15 min after induction or infection) in the untreated culture.

[b] Lipiarmycin was present from time zero of infection at 1 μg/ml.

[c] Rifampin was present from time zero of induction or infection at 5 μg/ml.

[d] Burst size of φ105 wild type was determined after induction by mitomycin C (see legend to Fig. 3).

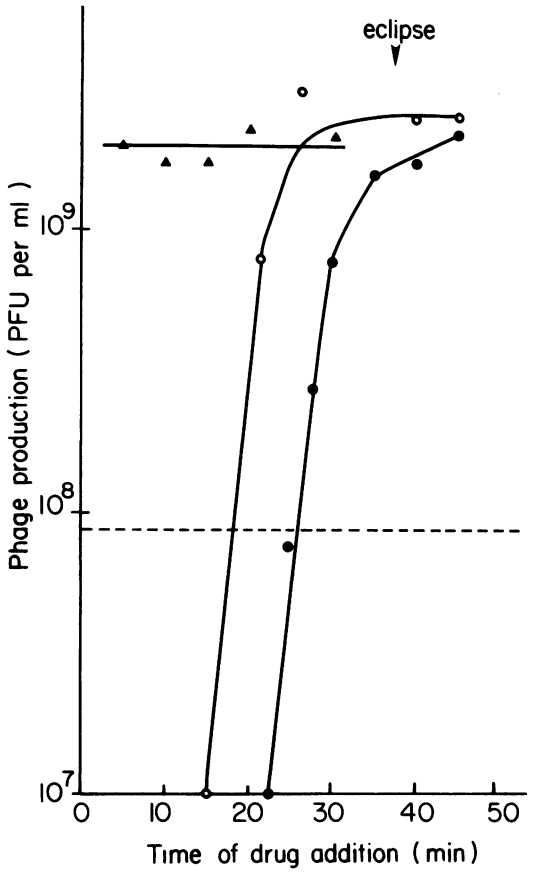

FIG. 1. *Effect of lipiarmycin and rifampin on growth of SP01. Strains 3610 and LS105 were grown at 37°C in modified 121A medium (supplemented with 20 μg of adenine, leucine, and methionine per ml for LS105) to 3 × 10^8 cells/ml (Klett = 130). Cells were infected with phage SP01 at time zero (multiplicity of infection = 0.3); lipiarmycin (1 μg/ml) or rifampin (1 μg/ml) was added to samples of infected cells at the times indicated. Ten minutes after infection, cultures were diluted 10^4-fold into the same medium (with or without lipiarmycin or rifampin, as appropriate). All samples were plated with indicator cells at 110 to 120 min after infection. The dashed line indicates the titer of infective centers determined at 12 min after infection of cells that had not received drugs. The eclipse period in untreated infected cells was determined by titering intracellular phage, as described in Materials and Methods; the arrow indicates the time at which the intracellular phage titer equaled 1 PFU per infected cell. The points shown for strain 3610 are from a single experiment. Four other experiments gave equivalent results. Symbols: (○) effect of lipiarmycin on SP01 infection of 3610; (●) effect of rifampin on SP01 infection of 3610; (▲) effect of lipiarmycin on SP01 infection of LS105.*

the growth of φ105 in the rifampin-sensitive host 3610 and its rifampin-resistant derivative LS2. The burst size of the clear-plaque mutant φ105c30 in strain 3610 was drastically reduced when rifampin was added before 30 min after infection (Fig. 3A), as was the case when the

TABLE 3. *Effect of lipiarmycin and rifampin on SP01-modified RNA polymerases in vitro*[a]

Enzyme[b]	Drug[c]	RNA synthesized (nmol/10 min)	Residual activity (%)
Host holoenzyme		1.14	100
(3.9 μg)	Lpm	0.4	35
	Rif	0.04	4
SP01 enzyme B		0.13	100
(1.3 μg)	Lpm	0.13	100
	Rif	0.01	8
SP01 enzyme C		0.21	100
(1.15 μg)	Lpm	0.20	95
	Rif	0.02	10
Host core enzyme		0.13	100
(6.5 μg)	Lpm	0.10	77

[a] RNA polymerase preparations listed were assayed with SP01 DNA as template as described in the text.
[b] For the amounts of enzymes used, RNA synthesis was linearly dependent on enzyme concentration.
[c] When added, lipiarmycin and rifampin were at 1 μg/ml.

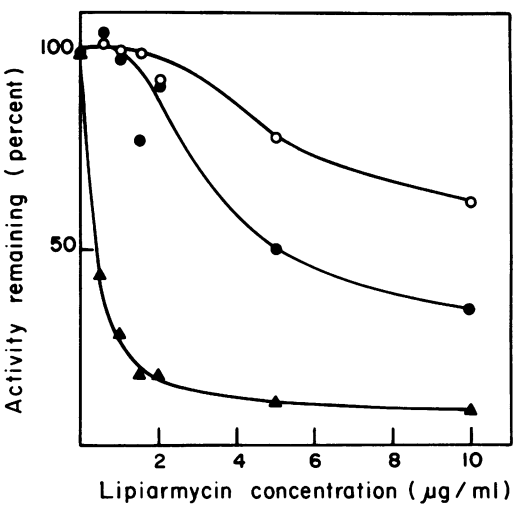

FIG. 2. *Effect of lipiarmycin concentration on SP01-modified RNA polymerases in vitro. Sensitivity to lipiarmycin of holoenzyme from strain 3610 and enzymes B and C from SP01-infected cells of 3610 was determined in RNA polymerase assays described in Materials and Methods with SP01 DNA as template. In the absence of lipiarmycin, enzyme B (2.6 μg/assay; ●), enzyme C (2.3 μg/assay; ○), and holoenzyme (6.5 μg/assay; ▲) incorporated 7,481, 7,309, and 23,708 cpm of [^{14}C]AMP, respectively. RNA synthesis, under these conditions, was linearly dependent on enzyme concentration.*

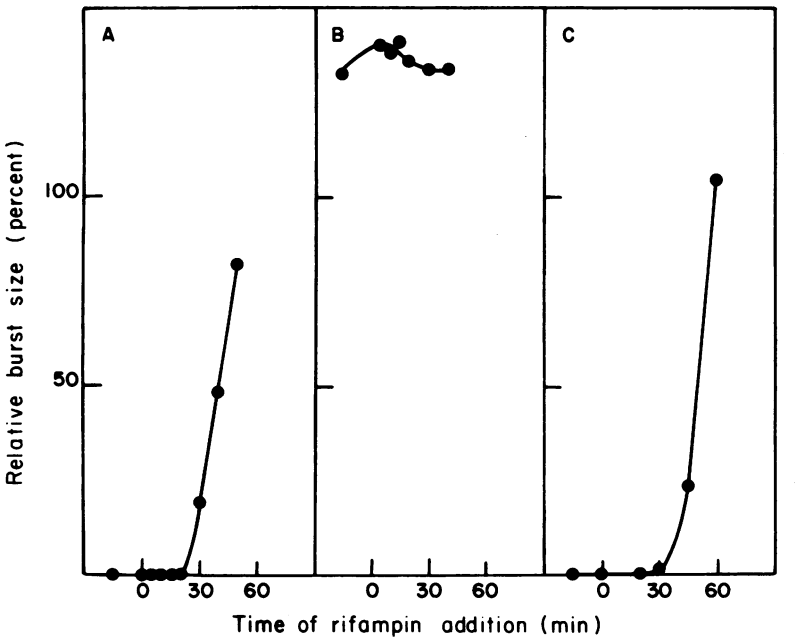

FIG. 3. *Effect of rifampin on growth of φ105. (A and B) Cultures of strains 3610 (A) and LS2 (B) were grown at 37°C in 121A medium to a density of 5 × 10⁷ cells/ml (Klett = 50) and infected with φ105c30 (multiplicity of infection = 1). After 1 min of adsorption, infected cells were diluted 1,000-fold and incubated further at 37°C. (C) The lysogenic strain 168(φ105) was grown in DSM medium to a density of 5 × 10⁷ cells/ml and treated with 0.5 μg of mitomycin C per ml (time zero of induction). After 10 min, induced cells were washed, suspended in fresh medium, and incubated at 37°C. Rifampin (5 μg/ml) was added in all cases at the times indicated. Phage bursts were determined at 90 min after induction or infection and were calculated as the phage titer at 90 min divided by the titer of infective centers. Actual burst sizes in the absence of drug are given in Table 2.*

drug was added before 30 min after induction of wild-type phage (Fig. 3C). However, infection was completely resistant to the drug in the rifampin-resistant host LS2, no matter when the drug was added (Fig. 3B), indicating that the sensitivity of φ105 growth to rifampin depends on the sensitivity of the host RNA polymerase. Although the time required for completion of phage RNA synthesis in φ105-infected cells is not known, the eclipse period of φ105c30 in strain 3610 was 35 min. It is therefore likely that phage transcription was completed by 30 min after infection, which would explain the relative resistance of infection to rifampin addition at later times. This result suggests that the phage does not code for its own RNA polymerase. The possibility remains, however, that the phage modifies the host enzyme, provided that the rifampin-sensitive component of the host polymerase is conserved.

In Fig. 4 the effects of treatment of φ105c30-infected cells with rifampin (2 μg/ml) and lipiarmycin (2 μg/ml) are compared. Either drug inhibited phage growth in the drug-sensitive strain 3610 if added early in infection. φ105 production was resistant to lipiarmycin in the drug-resistant host LS105, even when the drug was added at time zero (Table 2). The kinetics of development of resistance to lipiarmycin are not easily explained. Average phage production in the presence of lipiarmycin surpassed 1 PFU per infected cell when the drug was added at 17 min after infection; rifampin was more inhibitory than that until almost 25 min after infection. Although this might be taken as evidence for a phage-modified form of RNA polymerase active at late times, such an enzyme could not be the only form of RNA polymerase necessary for phage development, since complete lipiarmycin resistance was not achieved until the time at which infection became totally resistant to rifampin (compare the lipiarmycin curves of Fig. 1 and Fig. 4).

PBS2. Phage PBS2 induces the synthesis of at least two classes of RNA during its infection cycle (1). Only early RNA appears, however, in cells pretreated with chloramphenicol, indicating that protein synthesis after infection is required for transcription of late, but not early, genes. Both classes of RNA are synthesized in the presence of rifampin (even in rifampin-sensitive host cells); phage production is nearly

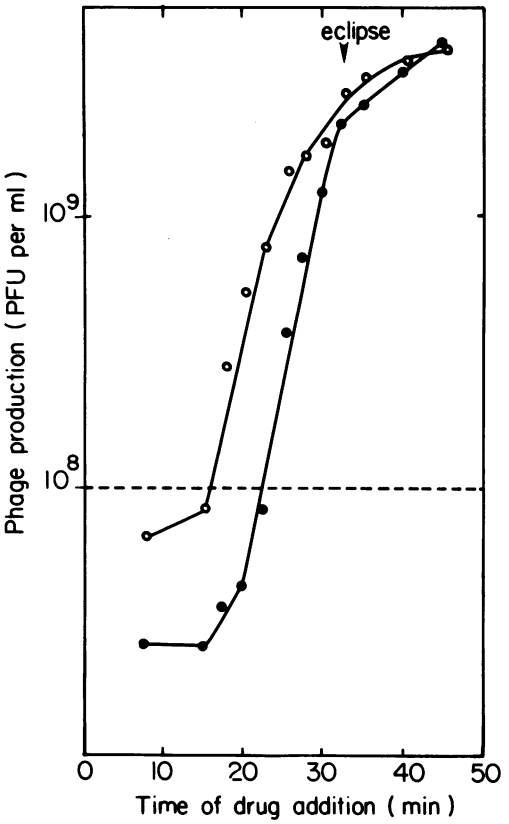

FIG. 4. *Effect of lipiarmycin and rifampin on growth of φ105. Strain 3610 was grown at 37°C in DSM medium to a density of 3 × 10^8 cells/ml and infected with phage φ105c30 (multiplicity of infection = 0.3). Four minutes after infection, the culture was diluted 10^4-fold in prewarmed DSM medium. Lipiarmycin or rifampin (2 μg/ml) was added to samples of the infected cultures at the times indicated. All samples were plated with indicator cells at 80 to 95 min after infection. The dashed line indicates the titer of infective centers determined at 10 min after infection of cells that had not received either drug. The arrow marked "eclipse" indicates the time at which the intracellular phage titer equaled 1 PFU per infected cell. The points shown are from a single experiment. Five other experiments gave comparable results. Symbols: (○) effect of lipiarmycin on φ105c30 infection; (●) effect of rifampin on φ105c30 infection.*

normal when rifampin is present at high concentrations throughout the infection cycle (15, 17). These observations suggest that PBS2 genes are transcribed by two forms of RNA polymerase: an early gene RNA polymerase, all of whose polypeptide constituents exist before infection, and a late gene RNA polymerase, which must be synthesized de novo after infection. Both enzymes must be resistant to rifampin. In fact, a rifampin-resistant PBS2-specific RNA polymerase that transcribes late genes in vitro has been purified from cells at late times of infection (1). Two hypotheses may explain the activity of the early RNA polymerase (1, 15, 17): (i) a phage-specific RNA polymerase is contained within the virion and is injected into the cell with the viral nucleic acid, as is the case for a coliphage, N4 (3, 13), or (ii) one or more polypeptides injected with the phage DNA modify host RNA polymerase, rendering it resistant to rifampin and competent to transcribe early phage genes. We have used lipiarmycin to try to distinguish between these two models.

We studied the effect of lipiarmycin on phage production in PBS2-infected wild-type cells and found that phage yield was greatly reduced when the drug was added (to 5 μg/ml) early in infection, but was unaffected by drug addition at late times (Fig. 5 and 6). Reduction in phage yield by drug addition at early times was due to slower and lower production of phage particles rather than to inhibition of phage release, since measurements of intracellular phage production followed the same pattern as that shown in Fig. 6 (data not shown). Drug concentrations lower than 5 μg/ml (e.g., 2.5 μg/ml) had a less dramatic effect on phage production (data not shown). These results suggest that the RNA polymerase active at early times is more sensitive to lipiarmycin than is the late enzyme, but is more resistant to lipiarmycin than is host holoenzyme. The late phage polymerase has been shown to be highly resistant to the drug in vitro (S. Clark, personal communication).

We found that PBS2 infection was much less sensitive to lipiarmycin in an Lpm^r mutant host

TABLE 4. *Burst size of PBS2 with or without drug treatment in various host strains*[a]

Host strain	Drug added[b]	Burst size at:	
		90 min	120 min
3610	None	40	41
	Lipiarmycin	2	7
	Rifampin	15	19
LS121	None	19	36
	Lipiarmycin	12	22
	Rifampin	2	3
	Lipiarmycin, rifampin	1	3
LS1213	None		30
	Lipiarmycin		2
	Rifampin		22

[a] Burst sizes were measured as PFU at 90 min or 120 min divided by the number of infective centers at 15 min after infection of the untreated culture. The numbers given are the averages of three to six experiments.

[b] Lipiarmycin or rifampin (or both) was added to 5 μg/ml at 7.5 min before infection.

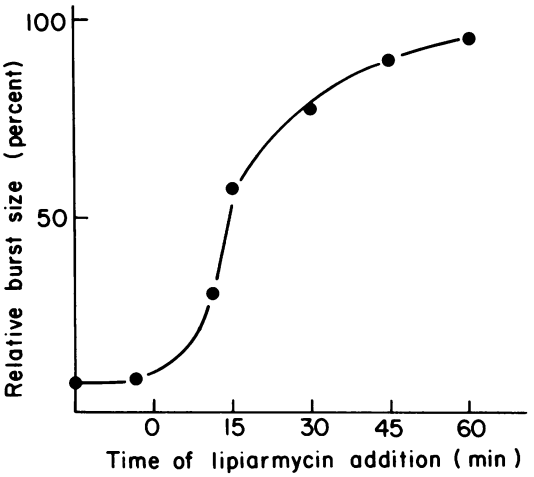

FIG. 5. *Inhibition by lipiarmycin of phage PBS2 in strain 3610. Wild-type (3610) cells were grown at 32°C in modified 121A medium to Klett = 150. At 15 min before infection, the culture was divided into 2-ml aliquots. At time zero, each aliquot was infected with PBS2 (multiplicity of infection = 1); lipiarmycin was added to 5 μg/ml at the times indicated before or after infection. After 10 min of adsorption at 32°C (5 min without shaking and 5 min with shaking), infected cells were diluted 10,000-fold into the same medium with or without lipiarmycin and grown at 32°C. Infected cultures were titered for phage bursts at 120 min after infection. Relative burst size (percentage of burst without lipiarmycin) is plotted as a function of time of drug addition. The burst size in the absence of lipiarmycin was 55.*

strain (LS121) than it was in wild-type host cells (Fig. 6B and Table 4). (It was necessary to use LS121 for these experiments because PBS2 does not adsorb to LS105 or other derivatives of JAS5. Strain LS121, like LS105, has a core mutation responsible for its Lpm^r phenotype [H. B. Alexander and A. L. Sonenshein, unpublished results].) Inhibition by lipiarmycin of PBS2 growth in wild-type cells thus seemed to be due to the sensitivity of host RNA polymerase. Further evidence for the involvement of the host enzyme in PBS2 early transcription came from our observation that, although resistant to lipiarmycin, PBS2 growth in strain LS121 became sensitive to rifampin and to the combination of rifampin and lipiarmycin (Fig. 6B). In strain LS1213, an Rfm^r derivative of LS121, infection by PBS2 regained resistance to rifampin, although resistance to lipiarmycin was lost (Fig. 6C). PBS2 growth in the wild type and in both mutant strains was resistant to streptolydigin (data not shown).

The mutations conferring Lpm^r and Rfm^r to strains LS121 and LS1213 were clearly lesions affecting components of host RNA polymerase, since RNA polymerase activity in extracts of strain LS121 was resistant to lipiarmycin and in extracts of strain LS1213 was highly resistant to both lipiarmycin and rifampin (Fig. 7). These mutations were mapped by transformation into strain DR10, an Std^r mutant of W168. The mutation *lpm-121* was 93% linked with *rfm-12* and

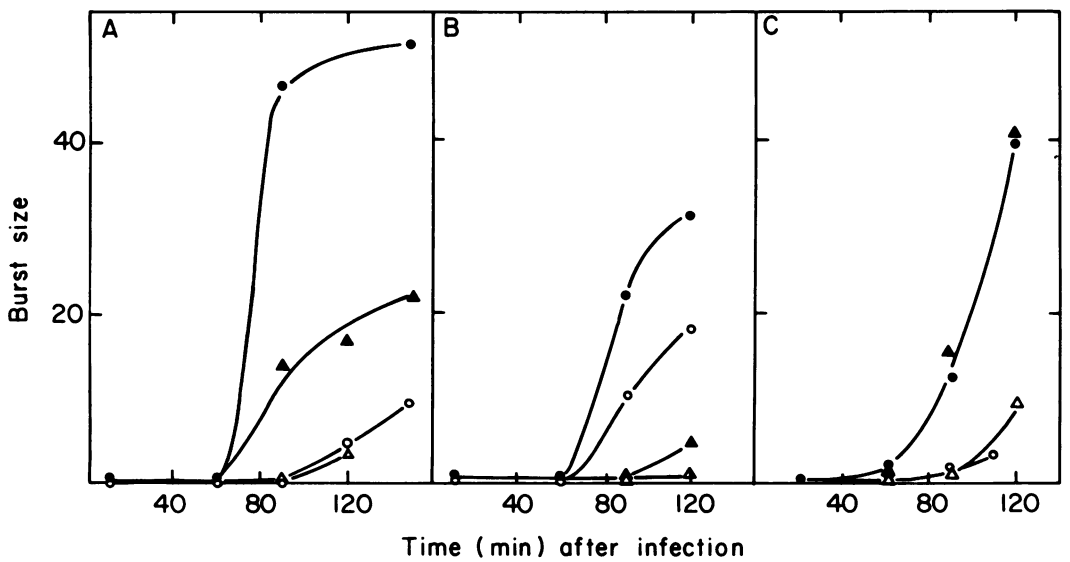

FIG. 6. *Effect of lipiarmycin and rifampin on single-step growth of PBS2 in strains 3610, LS121, and LS1213. Cultures of strain 3610 (A), LS121 (lpm-121) (B), and LS1213 (lpm-121 rfm-12) (C) were grown and infected as described in the legend to Fig. 5. Lipiarmycin (5 μg/ml) or rifampin (5 μg/ml) was added to cultures 7.5 min prior to phage infection. Burst sizes determined in additional experiments are summarized in Table 4. Symbols: (●) no drug added; (○) lipiarmycin added; (▲) rifampin added; (△) lipiarmycin and rifampin both added.*

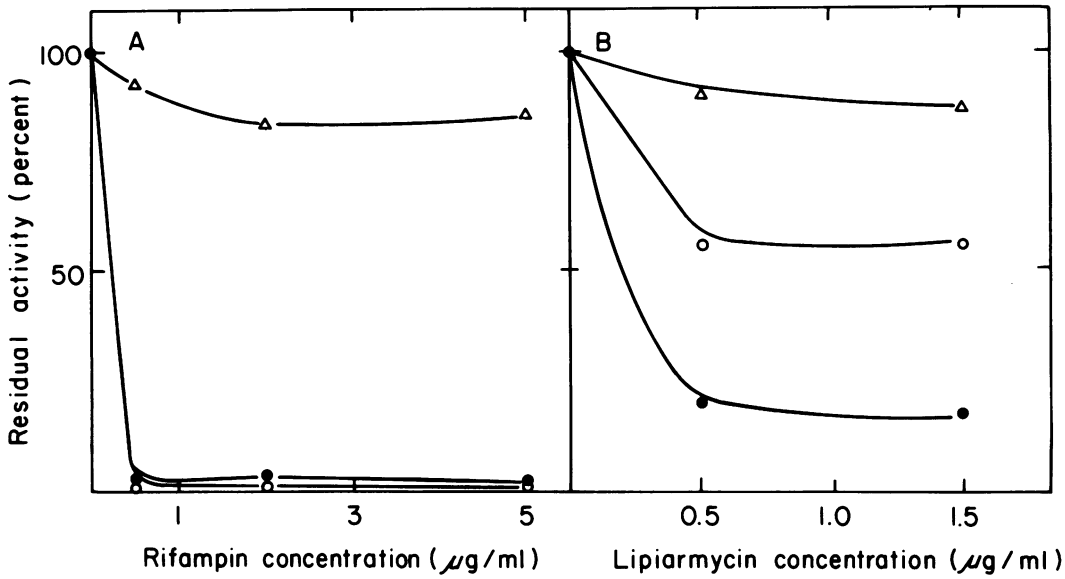

FIG. 7. *Drug sensitivity of RNA polymerase activity in wild-type and mutant strains. Strains 3610, LS121, and LS1213 were grown in medium 121A to midlogarithmic phase. Harvested cells were broken by sonication, and RNA polymerase was purified partially by precipitation with ammonium sulfate as described (10), except that phenylmethylsulfonyl fluoride (400 μg/ml) was present in all buffers and the EDTA concentration was 3 mM. RNA polymerase activity was assayed with phage φe DNA as template in the presence and absence of rifampin as indicated. To measure sensitivity to lipiarmycin, extracts were incubated with lipiarmycin at the concentrations indicated for 2 min at 37°C and then diluted 10-fold into reaction mix. In the absence of either drug, the amounts of extract used caused incorporation of 3,700 (3610), 6,200 (LS121), and 4,600 (LS1213) cpm. Symbols: (●) 3610; (○) LS121; (△) LS1213.*

std-10. By transduction with phage PBS1, *lpm-121* was 98% linked to *rfm-11*.

In summary, we found that mutational alteration of host RNA polymerase caused alterations in the sensitivity of PBS2 growth to lipiarmycin and rifampin. Taken together, these results suggest that PBS2 infection has a previously undetected dependence on host RNA polymerase. This is likely to be due to the involvement of one or more host RNA polymerase components in transcription of early phage genes (although it cannot be excluded that transcription of unknown host genes required for successful infection might be responsible for the dependence of PBS2 growth on host RNA polymerase). Modification of host RNA polymerase by a phage protein injected along with phage DNA could account for our observations and those of others. Such a modification might fortuitously render the RNA polymerase resistant to rifampin (possibly by making the binding site inaccessible) while enabling it to transcribe early phage genes (RNA polymerase from uninfected cells does not transcribe PBS2 DNA in vitro; 1). The modified RNA polymerase would have a sensitivity to lipiarmycin that is intermediate between that of host holoenzyme and the "late" phage polymerase. Since this sensitivity is determined at least partly by a host subunit, mutations in the gene for that subunit (e.g., *lpm-121*) could render the enzyme and, as a consequence, PBS2 infection resistant to lipiarmycin. In the case of strain LS121, the mutation to Lpm^r would interfere with the normally rifampin-resistant character of the modified RNA polymerase. To explain the properties of strain LS1213, we postulate that the combination of two host RNA polymerase mutations and a phage-induced modification produce an early phage gene-transcribing enzyme that is as sensitive to lipiarmycin as is the enzyme in wild-type-infected cells. We are now measuring directly the effect of lipiarmycin on early and late PBS2 RNA synthesis and trying to identify a modified form of host RNA polymerase that would be able to transcribe early genes.

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