Transcription During the Development of Bacteriophage ϕ 29: Production of Host- and ϕ 29-Specific Ribonucleic Acid

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The synthesis of ribonucleic acid (RNA) during development of the virulent *Bacillus subtilis* bacteriophage ϕ 29 has been analyzed. Transcription of host deoxyribonucleic acid (DNA) continues at the preinfection rate throughout the latent period of viral growth. RNA-DNA hybridization was used to show that host messenger RNA synthesis continues late into the phage lytic cycle. Amino acid-labeling experiments show that this RNA is continuously used to produce protein. Ribosomal RNA production is not inhibited by phage infection. Small quantities of phage-specific RNA first appear between min 6 and 9 after infection. This RNA is made exclusively from one of the ϕ 29 DNA strands. At 12 min postinfection, when phage DNA replication commences, large quantities of viral RNA start to be synthesized. This RNA appears to be transcribed from both strands of ϕ 29 DNA. Studies with rifamycin and rifamycin-resistant host strains showed that the production of all phage ϕ 29-specific RNA requires those components of the host RNA polymerase which are sensitive to this antibiotic. Thus, phage ϕ 29 does not stop transcription of host DNA and may produce only one element for regulation of transcription of its own DNA. These findings may reflect the limited amount of genetic information carried by this phage.

Bacteriophage ϕ 29 is a small *Bacillus subtilis* phage offering several advantages for studies of viral development (3, 14, 35). One interesting feature of ϕ 29, its structural complexity (3), requires that a large portion of its deoxyribonucleic acid (DNA) specifies structural proteins. Recent studies have shown that from 45% (Hawley, *in preparation*) to 60% (1, 21) of the ϕ 29 genome of molecular weight 11×10^6 (4) codes for seven proteins found in purified particles. Thus, the number of ϕ 29 gene products having regulatory functions during viral development is likely to be low in comparison to more complex phages. This premise is supported by results obtained in temperature-shift experiments with various temperature-sensitive (ts) mutants of phage ϕ 29. By shifting ϕ 29 ts mutants from permissive to non-permissive conditions at various intervals after infection, it was demonstrated that only two or three ϕ 29 genes are expressed "early" or prior to ϕ 29 DNA replication (27, 34). Thus, in contrast to larger phages such as *B. subtilis* phage SPO1 and *Escherichia coli* phage T4, whose transcription programs are complex (5, 6, 10, 11, 26), the regulation of transcription of phage ϕ 29 DNA may be relatively simple.

As a part of our studies of ϕ 29 transcription, we present here an analysis of host and viral ribonucleic acid (RNA) production. Infection with phage ϕ 29 has little effect on the production and utilization of host RNA. The production of viral-specific RNA occurs first from one of the ϕ 29 DNA strands, and, at the onset of viral DNA replication, both DNA strands are transcribed. The production of all ϕ 29 RNA requires those components of the host RNA polymerase which are sensitive to the antibiotic rifamycin.

MATERIALS AND METHODS

Bacteria and phage. *Bacillus amyloliquefaciens* H (38) was used for the preparation of RNA from ϕ 29-infected cells. *B. subtilis* 12A, an asporogenous mutant of *B. subtilis* 168M, is the normal plating host for phage ϕ 29. The rifamycin-resistant mutants, RFR 42 and RFR 43, were obtained from E. P. Geiduschek (12). Phage ϕ 29 is the wild-type virus originally isolated by B. E. Reilly (25).

Growth conditions. Growth of bacteria in Penassay Broth (Difco, Detroit, Mich.) and infection with phage ϕ 29 have been described in detail (4, 14, 28). For labeling of proteins with ^{14}C -amino acids, the bacteria were grown and infected in the minimal medium of Spizizen (31) supplemented with 0.01% yeast extract

(Difco), 0.02% tryptone (Difco), and tryptophan (11 μ g/ml). The burst size of ϕ 29 on various hosts was determined as described elsewhere (27).

Measurement of RNA synthesis. Total RNA production in infected and uninfected cultures was measured by the addition of carrier-free $\text{H}_3^{32}\text{PO}_4$ (New England Nuclear Corp., Boston, Mass.) to a final concentration of 5 $\mu\text{Ci/ml}$. Fractionation of bacteria and quantitation of ^{32}P -labeled RNA was performed as described elsewhere (29). Alternatively, RNA accumulation was determined by the addition of ^3H -uridine (New England Nuclear Corp.) to a final concentration of 2 μCi per 10 μg per ml. To measure metabolically stable RNA (11), cells were labeled with ^3H -uridine and then treated with rifamycin (generously supplied by E. P. Geiduschek) at a concentration of 2 $\mu\text{g/ml}$ for 14 min at 37 C. The trichloroacetic acid-insoluble counts remaining after this treatment were determined as described below and were taken as the stable RNA fraction. When the rate of RNA synthesis was being measured, 1- or 3-min pulses with ^3H -uridine (1 to 2 $\mu\text{Ci/ml}$) were terminated by the addition of an equal volume of ice-cold 10% trichloroacetic acid. After 30 min at 4 C, the samples were collected over 25-mm membrane filters (0.45 μm pore size, HA type, Millipore Corp.) and washed with cold 5% trichloroacetic acid. The filters were dried, and the retained radioactivity was determined as described below.

^3H labeling and isolation of RNA. RNA used in the hybridization studies was prepared from infected or uninfected broth cultures which had been pulse-labeled for 3 min by the addition of ^3H -labeled uridine (27.8 Ci/mmol, New England Nuclear Corp.) to a concentration of 40 to 100 $\mu\text{Ci/ml}$. Incorporation was terminated by pouring the culture onto frozen 0.01 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride buffer (pH 7.5) containing MgCl_2 (0.005 M), chloramphenicol (200 $\mu\text{g/ml}$), and sodium azide (0.01 M). Cells were collected at 4 C by centrifugation (10,000 $\times g$, 15 min). RNA was extracted from the cells by hot phenol as described by Bolle et al. (5). The ^3H -labeled RNAs had specific activities of 4×10^3 to 96×10^3 counts per min per μg of RNA. Exposure to 40 μg per ml of boiled pancreatic ribonuclease (7) in 2X standard saline-citrate (SSC: 0.15 M NaCl, 0.015 M sodium citrate) for 60 min at 25 C or treatment with 0.3 N KOH at 30 C for 24 hr reduced the acid-precipitable ^3H -activity of the RNA by greater than 99%. RNA yields were about 3 mg per 10^{10} bacteria.

Preparation of DNA. Phages purified on CsCl (3) were treated with 8 M urea at 25 C for 20 min. After overnight dialysis at 4 C against SSC, nuclease-free Pronase (Calbiochem, Los Angeles, Calif.) was added to a concentration of 1%, Sarkosyl NL-30 (Geigy Industrial Chem., Ardsley, N.Y.) was added to a final concentration of 1%, and the mixture was incubated at 37 C for 24 hr. The DNA was extracted three times with SSC-saturated phenol and dialyzed into 0.01 M Tris-hydrochloride (pH 7.4) at 4 C.

The method of Anagnostopoulos and Spizizen (2) was used to prepare *B. amyloliquefaciens* H DNA. The DNA was dialyzed into Tris-hydrochloride buffer as described above.

Preparation of the heavy and light strands of ϕ 29

DNA. The intact complementary strands of ϕ 29 DNA were prepared as described previously (22). After annealing at 65 C for 120 min, the isolated strands were diluted with 6X SSC and placed on filters (1 to 2 μg of DNA per filter) for hybridization as described below.

RNA-DNA hybridization. Annealing of ^3H -labeled RNA to various DNAs immobilized on nitrocellulose filters was performed as described by Bøvre and Szybalski (7). The rapid renaturation of the relatively small ϕ 29 DNA single strands (molecular weight 5.5×10^6) in high salt concentrations restricted use of the liquid hybridization methods of Nygaard and Hall (23). DNA samples were heated (97 C for 10 min), rapidly chilled on ice, and loaded onto Bac-T-Flex type B6 filters (Schleicher & Schuell Comp., Keene, N.H.) with 6X SSC by employing a multiple-sample filtration apparatus (DNA 40, New Brunswick Scientific Corp., New Brunswick, N.J.). Filters were baked at 80 C in a vacuum oven for 3 hr and stored at room temperature. Hybridizations were performed in 1.3 ml of hybridization fluid (one volume of 2X SSC and one volume of phenol-saturated 2X SSC, pH 7.5) for 24 hr at 66 C. Ribonuclease (pancreatic ribonuclease, type 1-A, Sigma Chemical Comp., St. Louis, Mo.) treatment was performed in 2 ml of 2X SSC at a concentration of 20 $\mu\text{g/ml}$ for 60 min at room temperature. Accessory hybridization data is presented in the legends to Fig. 2 and 3.

Labeling of proteins with ^{14}C -amino acids. Proteins in infected and uninfected cells were pulse-labeled for 3 min at 37 C (1 ml of cells, 2×10^6 cells/ml) by the addition of a ^{14}C -amino acid mixture (2 $\mu\text{Ci/ml}$, 230 $\mu\text{Ci/mm}$, New England Nuclear Corp.). The pulse was terminated by pouring the cells over a frozen salt solution which contained 0.1 M NaCl, 0.05 M sodium citrate, 0.01 M sodium azide and 200 μg of chloramphenicol per ml. The cells were immediately sedimented at 4 C (16,300 $\times g$, 5 min) and suspended in the same salt solution containing egg-white lysozyme (30 $\mu\text{g/ml}$, Calbiochem). After cell lysis, a drop of chloroform was added, followed by MgSO_4 to 0.01 M and pancreatic deoxyribonuclease (20 $\mu\text{g/ml}$, Calbiochem). After incubation at 37 C for 30 min, the samples were centrifuged (20,000 $\times g$, 20 min) and the supernatant fluids were dialyzed against 0.06 M Tris-hydrochloride (pH 6.8) containing 0.1% sodium dodecyl sulfate (SDS).

Gel electrophoresis and autoradiography. The system for SDS-polyacrylamide gel electrophoresis was essentially that of Laemmli (19) except that a slab gel apparatus was used (32). Autoradiographs of dried gels were prepared by using Kodak No-Screen medical X-ray film, and microdensitometer tracings were made with a Joyce, Loeb MK III CS Microdensitometer.

Radioisotope counting procedures. Radioactive samples were counted with a Packard 2425 Tri-Carb liquid scintillation spectrometer as described previously (29).

RESULTS

RNA synthesis after phage ϕ 29 infection. Net accumulation of RNA was determined at various intervals following infection with phage ϕ 29

(Fig. 1). Phage infection has little effect on total RNA synthesis until 50 min, at the onset of massive cell lysis. The normal eclipse period for phage $\phi 29$ under the conditions of our experiments is 30 min (28). The rate of RNA synthesis in $\phi 29$ -infected cells was analyzed by pulse-labeling with ^3H -uridine. The rate of RNA synthesis after infection with phage $\phi 29$ remains constant until very late in infection (Table 1). The lack of a marked inhibition of RNA synthesis by phage $\phi 29$ infection led us to examine the nature of the RNA species produced in infected cells.

Hybridization analysis of the RNA synthesized after $\phi 29$ infection. To differentiate between host and viral transcripts within infected cells, pulse-labeled RNA was isolated and analyzed by hybridization to bacterial and phage DNA. Essentially no RNA from uninfected host cells will

anneal to $\phi 29$ DNA (Table 2). In contrast, pulse-labeled RNA from phage-infected cells efficiently hybridizes to both host and phage DNA. Thus, it is possible to utilize RNA-DNA hybridization to detect and quantitate phage RNA in $\phi 29$ -infected cells. Figure 2 presents the results of an experiment in which pulse-labeled RNA was prepared at various intervals from phage $\phi 29$ -infected cells and hybridized to host and phage DNA. The percentage of the RNA which will anneal with host DNA remains constant until at least 33 to 36 min after infection. Since these hybridizations were not done under conditions of excess DNA over the abundant host RNA species, such as ribosomal RNA, the values obtained do not represent a true quantitation of host transcription. However, the lack of inhibition of host transcription by phage $\phi 29$ infection is apparent.

We have not yet detected $\phi 29$ -specific RNA prior to 6 min after infection. Between min 6 and 9 a low level of phage-specific RNA can be detected, and phage transcription consistently reaches a plateau level until about 15 min after infection. Between 12 and 15 min there is a rapid increase in phage transcription; this time coincides with the onset of $\phi 29$ DNA replication (28). Large quantities of $\phi 29$ -specific RNA are produced until cell lysis occurs. Since the hybridization to $\phi 29$ DNA was carried out at DNA excess, the data presented in Fig. 2 illustrate quantitative transcription of the $\phi 29$ DNA molecule during infection. At 33 to 36 min after infection, at least 10 times as much viral RNA is being produced in comparison to the 6- to 9- or 9- to 12-min intervals after phage addition.

FIG. 1. RNA synthesis in uninfected (○) and phage $\phi 29$ -infected (●) cultures of *B. amyloliquefaciens* H. The multiplicity of infection with phage $\phi 29$ was 10.

TABLE 1. Rate of RNA synthesis in uninfected and phage $\phi 29$ -infected *B. subtilis* 12A

Time of pulse (min)	^3H -uridine incorporated ^a		Uninfected/infected
	Infected ^b	Uninfected	
0-3	908	960	1.06
3-6	1,016	1,012	1.00
6-9	1,108	1,064	0.96
9-12	1,148	1,160	1.01
12-15	1,116	1,392	1.25
15-18	992	1,228	1.24
24-27	1,160	2,328	2.01
33-36	844	3,768	4.46

^a Counts per minute per milliliter of culture. Cells were pulse-labeled with ^3H -uridine at a final concentration of 4 $\mu\text{g}/\text{ml}$ (0.125 $\mu\text{Ci}/\mu\text{g}$).

^b Cells at a concentration of $5 \times 10^7/\text{ml}$ were infected at 0 time with a multiplicity of infection of 10.

TABLE 2. Hybridization specificity of ^3H -RNA from phage ϕ 29-infected and uninfected *B. subtilis* 12A

Source of RNA	Percent hybridization	
	<i>B. subtilis</i> DNA ^a	ϕ 29 DNA ^b
Uninfected cells (3-min pulse)	4.63	0.04
Infected cells (6- to 9-min pulse) (24- to 27-min pulse)	4.92	1.32
	4.10	16.20

^a Host DNA concentration was 5 μg per filter.

^b Phage DNA concentration was 2 μg per filter.

FIG. 2. Hybridization of pulse-labeled RNA from phage ϕ 29-infected cells to *B. amyloliquefaciens* H DNA (open bars) and phage ϕ 29 DNA (solid bars). Host DNA was used at a concentration of 13 μg per filter, and phage DNA was at a concentration of 10 μg per filter. RNA was used at a concentration of 3 μg (4.8 to 12.3×10^8 counts per min per μg). Maximum background hybridization in the absence of DNA was 16 counts per min.

Previous preliminary studies (22) demonstrated that both of the complementary strands of phage ϕ 29 DNA were transcribed during the development of this virus. Figure 3 presents the results of an experiment where pulse-labeled RNA prepared at various intervals from phage ϕ 29-infected cells was hybridized to self-annealed preparations of the heavy (H) and light (L) strands of ϕ 29 DNA. Until min 12 after phage infection, almost all of the viral RNA is made from the L strand. Near min 12 after infection there is a great increase in transcription from the H DNA strand. At the same time, reading of the L strand also increases. At 33 to 36 min after infection, approximately 73% of the viral transcripts are being made from the H strand. The nature of the RNA species made from the L and H strands after 12 min of infection have been studied, and the results of this work will be presented elsewhere (Schachtele et al., *in preparation*).

FIG. 3. Hybridization of pulse-labeled RNA from phage ϕ 29-infected cells to the isolated L (open bars) and H (solid bars) strands of ϕ 29 DNA. DNA was at a concentration of 2 μg per filter. The RNA preparations were the same as those for Fig. 2.

Production and translation of host RNA following phage ϕ 29 infection. The observations in the preceding sections indicated that phage ϕ 29 infection may not affect either stable or messenger RNA (mRNA) transcription from the host chromosome. Gage and Geiduschek (11) have used the antibiotic rifamycin to measure synthesis of 4, 5, 16, and 23S *B. subtilis* RNA in phage SPO1-infected cells. Incubation of infected cells with rifamycin allowed the degradation of unstable RNA species, whereas metabolically stable RNA remained unaffected. Figure 4 presents an experiment where uninfected and ϕ 29-infected cells were pulse-labeled for 1 min with ^3H -uridine at various times and then incubated with rifamycin before measuring the amount of RNA present. Accumulation of stable *B. subtilis* RNA is only slightly affected by infection with phage ϕ 29. The amount of RNA degraded in the presence of rifamycin was consistently 18 to 23% of the total RNA in each sample. In additional experiments (data not shown) it was demonstrated that the RNA remaining after rifamycin treatment could be isolated after protoplast formation (11) and banded in a 5 to 20% sucrose gradient in the regions corresponding to 4, 16, and 23S as determined with purified RNAs obtained from Miles Laboratories, Inc. (Elkhart, Ind.).

Translation of host mRNA occurs throughout the phage ϕ 29 lytic cycle. Figure 5 demonstrates the labeling of host proteins with ^{14}C -amino acids 30 to 33 min after phage infection. About eight ϕ 29-specific proteins can be distinguished in the autoradiograph from infected cells, including the three major ϕ 29 structural proteins. Thus about half of the phage-induced proteins are masked by the large number of labeled host proteins still being made at the end of the latent period of phage development. Although there are definite decreases in the amount of some of the host protein bands when ϕ 29-infected cells are compared to uninfected controls, large numbers of host proteins are made throughout the phage lytic cycle. Analysis of ϕ 29 proteins formed in ultraviolet-irradiated cells will be presented elsewhere (Hawley, *in preparation*).

Effect of rifamycin on phage ϕ 29 development. Rifamycin is a potent inhibitor of RNA polymerase activity (30, 36, 37) and has thus been used to study the role of the drug-sensitive part of the polymerase enzyme in the development of various bacterial viruses (12, 13, 33). Table 3 shows that growth of phage ϕ 29 on two rifamycin-sensitive strains of *B. subtilis* is completely inhibited by the presence of a small amount of rifamycin. In contrast, phage ϕ 29 can readily propagate in the presence of the drug in rifamycin-resistant mutants of *B. subtilis*. DNA-dependent RNA syn-

FIG. 4. Accumulation of stable RNA in uninfected and phage ϕ 29-infected cultures of *B. subtilis* 12A. Cells were labeled with ^3H -uridine (16 $\mu\text{g}/\text{ml}$, 0.025 $\mu\text{Ci}/\mu\text{g}$) for 3 min and one-half of the culture was infected with ϕ 29 at a multiplicity of 10. Samples were removed at various intervals and incubated with rifamycin (2 $\mu\text{g}/\text{ml}$) for 14 min prior to measuring the amount of labeled trichloroacetic acid-precipitable RNA present. Symbols: $\circ$, RNA in uninfected culture; $\bullet$, RNA in ϕ 29-infected culture.

thesis in extracts from mutant RFR 42 was previously shown to be resistant to rifamycin (12).

Figure 6 presents an experiment where rifamycin was added to samples of an infected culture at various intervals, and the effect of the drug was determined by measuring the yield of phage after subsequent incubation at 37 C for 60 min. The addition of rifamycin as late as 22 min after

FIG. 5. Microdensitometer tracings of autoradiographs of polyacrylamide gels in which ^{14}C -labeled proteins from ϕ 29-infected (A) and uninfected (B) *B. subtilis* were subjected to electrophoresis. Infected cells were pulse-labeled from 30 to 33 min after infection. The numbered peaks correspond to the phage ϕ 29 appendage (1), major head (2) and horn (3) proteins.

infection completely blocked phage ϕ 29 production. The ability to make phage in the presence of the antibiotic increased after 22 min of infection, and, at 40 min, addition of the drug had no effect on phage yield. Since rifamycin is capable of blocking RNA production in ϕ 29-infected cells within 1 or 2 min (see Fig. 7), it

would appear that synthesis of some transcript(s) essential for the production of completed phage have not been initiated prior to 22 min postinfection. By 40 min postinfection there is adequate RNA available to produce enough proteins to give a normal yield of phage.

Figure 7 presents an experiment where the

TABLE 3. Effect of rifamycin on phage $\phi 29$ production in various hosts

Host	Average burst size	
	With rifamycin ^a	Without rifamycin
<i>B. subtilis</i> 12A	1	520
168 M	1	312
RfR 42	240	284
RfR 43	122	117

^a Rifamycin was added at the time of infection at a concentration of 2 $\mu\text{g}/\text{ml}$.

FIG. 6. Effect of rifamycin on phage $\phi 29$ production in *B. amyloliquefaciens* H. Cells were infected with phage $\phi 29$ at a multiplicity of 10. One set of samples was removed at intervals and transferred to tubes containing rifamycin (2 $\mu\text{g}/\text{ml}$). After incubation at 37 C for 60 min, each tube was assayed for viable phage. A second set of samples was treated at identical intervals with chloroform and titered for phage after remaining at 4 C for 30 min. Symbols: $\circ$, rifamycin-treated samples; $\bullet$, chloroform-treated samples.

FIG. 7. Rifamycin inhibition of RNA synthesis in phage $\phi 29$ -infected *B. subtilis* 12A. A culture containing 2×10^8 cells per ml was infected with phage at a multiplicity of 10. At 1, 10, and 24 min after infection, portions of the culture were removed to flasks containing rifamycin (final concentration, 2 $\mu\text{g}/\text{ml}$). At various times, 1-ml samples of each culture were pulse-labeled for 1 min with ^3H -uridine (2 $\mu\text{g}/\text{ml}$, 0.5 $\mu\text{Ci}/\mu\text{g}$). Symbols: $\circ$, samples from control cultures; $\bullet$, samples from rifamycin-containing cultures.

effect of rifamycin on RNA synthesis at various times after infection with phage $\phi 29$ was determined. The production of RNA at 25 min after infection was as sensitive to rifamycin as RNA synthesis at earlier times in the phage lytic cycle. It would appear from this data and the previous observations that transcription of the entire phage $\phi 29$ genome is dependent on the presence of the rifamycin-sensitive part of the host RNA polymerase.

DISCUSSION

In marked contrast to the very effective blockage of *E. coli* macromolecular synthesis after infection with T-even phages (9, 17), the production of *B. subtilis* nucleic acids and protein

appears to be only slightly affected by infection with phage $\phi 29$. We had demonstrated previously (28) that $\phi 29$ infection had little effect on the increase in optical density of a culture until near the onset of cell lysis and that host DNA was replicated during the latent period of phage development. It would appear from these observations, and the data presented in this paper, that phage $\phi 29$ does not carry information for the production of elements needed to suppress host macromolecular synthesis. Investigations on other virulent *B. subtilis* phages have shown that there is a great variation in the extent of inhibition of host biosynthetic processes after infection (11, 15, 24, 39). In general, virulent *B. subtilis* phages do not show the very rapid shut-off of host macromolecular synthesis characteristic of the *E. coli* T-even phages (9). Although replication of host DNA in $\phi 25$ - (20), $\beta 22$ - (15), 2C- (39), and SPO1- (11) infected cells is blocked prior to or at the onset of viral DNA replication, transcription of host DNA often continues well into the latent period of viral development (11, 15). In phage SPO1-infected cells, the inhibition of host transcription is noncoordinate since host mRNA production is blocked more effectively than stable RNA synthesis (11). The production of large quantities of functional host mRNA (Fig. 2 and 5) and stable RNA (Fig. 4) late in the lytic cycle of phage $\phi 29$ makes infection with this virus more similar to infection with a temperate *B. subtilis* phage such as SPO2 (18).

Although a large proportion of the RNA made in $\phi 29$ -infected cells is not of viral origin, the presence of $\phi 29$ mRNA can be efficiently detected by the annealing of RNA to $\phi 29$ DNA immobilized on membrane filters. Our inability to detect phage-specific RNA prior to 6 min after infection may reflect a very low level of transcription of a small fraction of the $\phi 29$ DNA molecule. We are currently investigating this apparent slow initiation of $\phi 29$ transcription. We emphasize that the patterns of phage-specific RNA production presented in Fig. 2 and 3 are very reproducible and have been obtained with three different series of mRNA preparations. The finding that all of the phage RNA produced prior to $\phi 29$ DNA replication is made from the L DNA strand and that subsequent RNA production is from both of the DNA strands indicates that $\phi 29$ mRNA may be readily divided into "early" (produced prior to 12 min, from the L strand only) and "late" (produced after 12 min, from the H and L strands) transcripts. It might be predicted that $\phi 29$ would code for a positive control element necessary for "late" transcription. Direct evidence for modifica-

tion of the host RNA polymerase after infection with phage $\phi 29$ has recently been presented (Holland and Whiteley, Abstr. Annu. Meet. Amer. Soc. Microbiol., p. 244, 1972).

The development of *E. coli* phages T4 (13) and λ (33), and *B. subtilis* phages SPO1 (12) and $\beta 22$ (16), is sensitive to the rifamycins throughout infection. The growth of these phages in host mutants with a rifamycin-resistant RNA polymerase indicates that the rifamycin-sensitive part of this enzyme is required for transcription of the entire phage genome. The finding that phage $\phi 29$ will grow in the presence of rifamycin in a mutant of *B. subtilis* with a rifamycin-resistant RNA polymerase (Table 3) and the sensitivity to rifamycin of RNA production late in the phage lytic cycle (Fig. 7) indicate that $\phi 29$ production is also dependent upon the host RNA polymerase during its development. The observation that the modified host RNA polymerase in $\phi 29$ -infected cells is sensitive to rifamycin (Holland and Whiteley, Abstr. Annu. Meet. Amer. Soc. Microbiol., p. 244, 1972) supports our findings.

The results presented in this paper are consistent with the observation that there is a low number of "early" $\phi 29$ genes (27, 34). There appear to be no phage functions involved in the repression of host macromolecular synthesis. The low level of phage mRNA produced until min 12 of infection indicates that there is limited expression of $\phi 29$ DNA prior to phage DNA replication. The finding that the "early" mRNA is made exclusively from one of the $\phi 29$ DNA strands will allow us to study various properties of this RNA. The utilization of specific $\phi 29$ mutants (14) and RNA-DNA hybridization competition experiments (5, 11) should allow us to carry out a detailed analysis of the transcriptional program of this virus.

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