JOURNAL OF VIROLOGY, June 1979, p. 771-776
0022-538X/79/06-0771/06$02.00/0

Vol. 30, No. 3

Program of Bacteriophage gh-1 DNA Transcription in Infected *Pseudomonas putida*

JAMES F. JOLLY†

Department of Biochemistry, Michigan State University, East Lansing, Michigan 48824

Received for publication 16 November 1978

The program of transcription in phage gh-1-infected *Pseudomonas putida* was examined. It was found that the host *P. putida* RNA polymerase transcribes early RNA from the L strand of gh-1 DNA during the initial stages of infection. The host RNA polymerase is also undoubtedly responsible for transcription of complementary RNA late in the infectious cycle because complementary RNA was not transcribed when rifampin was added to the infected cell culture. The gh-1-induced RNA polymerase transcribes late RNA from the L strand of gh-1 DNA late in the infectious cycle. The *P. putida* RNA polymerase transcribed only early RNA from primarily the L strand of gh-1 DNA in vitro when the molar ratio of enzyme to gh-1 DNA was 0.5. When the molar ratio was 50 the *P. putida* RNA polymerase transcribed RNA from the H strand of gh-1 DNA as well as complementary RNA. The gh-1 RNA polymerase transcribed only the L strand of gh-1 DNA in vitro but transcribed both early and late RNA.

When the bacteriophage T7 infects *Escherichia coli*, the leftmost 20% of the phage genome is transcribed by *E. coli* RNA polymerase (8, 13, 17). This early region of T7 DNA appears to be transcribed as one large message which is then processed by RNase III into five smaller RNA species (9, 10, 12, 15). One of the early phage RNA species, the gene 1 product, codes for a T7 RNA polymerase (6, 20), which then transcribes the remainder of the phage genome. Early RNA synthesis as well as host RNA synthesis is shut off during T7 infection (4, 5, 21). This shutoff of RNA synthesis appears to be regulated by a modification of *E. coli* RNA polymerase after infection with bacteriophage T7 (27).

The *Pseudomonas putida* bacteriophage gh-1 is similar in size and shape to bacteriophage T7 (16). Upon infection the bacteriophage gh-1 induces a new RNA polymerase (24) which is similar in size and properties to the T7 RNA polymerase. The gh-1 RNA polymerase transcribes only gh-1 DNA in vitro and is resistant to the antibiotic rifampin (24).

The present paper describes the program of transcription during gh-1 phage infection. The novel feature of gh-1 transcription is that complementary phage RNA is synthesized late in infection. Because the synthesis of complementary RNA is inhibited by rifampin, it is undoubtedly made by the host *P. putida* RNA polymerase.

† Present address: The Biological Laboratories, Harvard University, Cambridge, MA 02138.

MATERIALS AND METHODS

Materials. Lysozyme, chloramphenicol, and bovine serum albumin were obtained from Sigma Chemical Co. Unlabeled ribonucleoside triphosphates and dithiothreitol were from P-L Biochemicals. ^{3}H-labeled CTP (26.2 Ci/mmol) and ^{3}H-labeled uridine (26.2 Ci/mmol) were purchased from New England Nuclear Corp. Polyuridylic acid-polyguanidylic acid [poly(U,G)] was from Miles Laboratories. RNase was purchased from Calbiochem, and DNase I was from Worthington Biochemical Corp. Nitrocellulose membrane filters were obtained from Schleicher and Scheull. Rifampin was the gift of Luigi Silvestri, Gruppo Lepetit, Inc., Milan, Italy.

Purification of bacteriophage and isolation of DNA. Bacteriophage gh-1 was purified from *P. putida* (ATCC 12633) lysates by the method of Lee and Boezi (16). gh-1 DNA was isolated by the method of Thomas and Abelson (23).

The strands of gh-1 DNA were isolated and purified by the procedure of Szybalski et al. (22) utilizing poly(U,G). Both strands of gh-1 DNA bound poly(U,G). The strands that formed the more dense complex with poly(U,G) was designated the H strand, whereas the complementary strand was designated the L strand. Each of the purified strands of gh-1 DNA was self-annealed for 6 h at 65°C to form duplex DNA molecules with any contaminating complementary strands before use in hybridization studies.

Purification of RNA polymerase. The gh-1-induced RNA polymerase was purified as previously described (24).

The *P. putida* RNA polymerase holoenzyme was purified by the method of Johnson et al. (14) with the following modifications: all buffers contained 15% glycerol (vol/vol). Cell disruption was accomplished by use of a French pressure cell. The 30% ammonium sulfate fractionation step and the second DEAE-cel-

lulose column chromatography step were omitted; instead, the final step in the purification was gel filtration using a Bio-Gel A-1.5m column (1.5 by 84 cm).

The RNA polymerase preparations were greater than 90% pure as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Preparation of competitor RNA. *P. putida* was grown as described by Lee and Boezi (16). After cell growth had reached the mid-logarithmic phase, gh-1 phage was added to a multiplicity of 5 to 10 PFU per cell. The infected cells were allowed to incubate at 33°C for various time intervals after infection. The infected cells were then poured onto an equal volume of crushed, cooled (−20°C) ice, and centrifuged. When present, chloramphenicol (400 μg/ml) was added 5 min before infection and competitor RNA was prepared from cells 5 min after infection. Total RNA was extracted by the method of Bøvre and Szybalski (2). *E. coli* competitor RNA was prepared from uninfected *E. coli* B in the same manner.

Preparation of ^{3}H-labeled RNA in vivo. *P. putida* cells were grown in media containing (in grams per liter): $NaHPO_4$, 6; KH_2PO_4, 3; NaCl, 8; NH_4Cl, 2; Casamino Acids, 5; glucose, 20; and trace amounts of $FeCl_3$, manganese acetate, and Na_2SO_4. After the cells had reached the mid-logarithmic growth phase, the cells were concentrated 10-fold by centrifugation and chilled to 4°C. gh-1 phage was added at a multiplicity of 5 to 10 PFU per cell. After 10 min at 4°C, the infected culture was diluted into prewarmed media (33°C). The time of dilution was taken to be the zero time of infection. RNA was pulse labeled with ^{3}H-labeled uridine (25 μCi/ml) during the time intervals indicated in the legends to the appropriate figures. The pulses were terminated by rapidly chilling the cells in an ice-water bath and collecting by centrifugation. RNA was extracted by the procedure described above for preparation of competitor RNA. Early RNA pulse-labeled in the presence of chloramphenicol was prepared in the same manner except chloramphenicol (400 μg/ml) was added to the media 5 min before infection and the RNA was pulse labeled from 0 to 5 min after infection.

Preparation of ^{3}H-labeled RNA in vitro. ^{3}H-labeled RNA synthesis was carried out in vitro by *P. putida* holoenzyme in a reaction mixture containing 50 mM Tris-hydrochloride (pH 8), 10 mM $MgCl_2$, 2.5 mM Mn Cl_2, 50 mM KCl, 1 mM dithiothreitol, 0.4 mM in each of the four ribonucleoside triphosphates, 0.5 mg of bovine serum albumin per ml, and 0.2 mCi of ^{3}H-labeled CTP per ml. The final specific activity of CTP was about 8×10^4 cpm/nmol. The reactions were begun by the addition of enzyme and incubated for 45 min at 30°C. DNase was added (10 μg/ml), and the mixture was incubated at 37°C for 15 min. The ^{3}H-labeled RNA was extracted twice with an equal volume of phenol, and the aqueous phase was dialyzed extensively against 2× SSC (SSC is 0.15 M NaCl and 0.015 M sodium citrate, pH 7). The procedure for ^{3}H-labeled RNA synthesis by the gh-1 RNA polymerase in vitro was the same except the reaction mixtures did not contain $MnCl_2$ and KCl which inhibit the gh-1 RNA polymerase. The conditions of RNA synthesis were such that the rate of reaction was proportional to enzyme concentration.

DNA-RNA hybridization. ^{3}H-labeled RNA was hybridized to the separate strands of gh-1 DNA as outlined by Bøvre and Szybalski (2). Hybridization of in vitro ^{3}H-labeled RNA to the separated strands of gh-1 DNA was accomplished with a 40% hybridization efficiency. The hybridization efficiency of in vivo ^{3}H-labeled RNA isolated 0 to 5 min after infection was less than 1% because of the presence of excess *P. putida* RNA. The hybridization efficiency of in vivo ^{3}H-labeled RNA isolated later during infection was about 20%. All hybridization experiments were repeated several times. Hybridization blanks (minus DNA) were less than 100 cpm. DNA was in excess during hybridization.

Hybridization competition. Hybridization competition was carried out by the method of Nygaard and Hall (19) as described by Bøvre and Szybalski (2). The hybridization mixtures contained (in a total volume of 0.5 ml): 10 μg of denatured gh-1 DNA, ^{3}H-labeled RNA (at least 10,000 cpm per hybridization mixture), varying amounts of competitor RNA, and a final salt concentration of 2× SSC. Hybridization was carried out at 65°C for 6 h. The hybridization efficiency was about 65% for in vitro ^{3}H-labeled RNA, 35% for in vivo RNA pulse labeled at late stages of infection, and less than 1% for in vivo RNA pulse labeled 0 to 5 min after infection. Each point on the hybridization competition curves represents an average of several determinations.

Detection of complementary RNA. Aliquots of ^{3}H-labeled RNA were incubated under annealing conditions at 65°C for 6 h and then chilled to 4°C. The self-hybridized RNA and aliquots of unhybridized ^{3}H-labeled RNA (to serve as controls) were digested with S1 nuclease at 45°C in an assay mixture (0.2 ml) containing 30 mM sodium acetate (pH 4.5), 1 mM $ZnSO_4$, 5% glycerol, and 50 μg of S1 nuclease. S1 nuclease was purified by the procedure of Vogt (25) through the DEAE-cellulose chromatography step. Digestion was continued until the control digestion mixtures no longer contained trichloroacetic acid-insoluble radioactivity and there was no further digestion in the experimental mixtures. The amount of ^{3}H-labeled RNA self-hybridized was at least 100,000 cpm. The amount of radioactivity remaining after digestion of the unhybridized ^{3}H-labeled RNA controls was less than 500 cpm.

RESULTS

Temporal specificity of gh-1 transcription in vivo. Phage RNA transcribed during the initial stage of infection is referred to as early RNA. RNA transcribed by bacteriophage gh-1-induced RNA polymerase later in infection is referred to as late RNA. Chloramphenicol limits transcription to early RNA because gh-1 RNA polymerase is not synthesized in the presence of chloramphenicol (24).

In hybridization competition experiments, ^{3}H-labeled early RNA, synthesized in the presence of chloramphenicol, was, as expected, fully competed by unlabeled RNA isolated from infected cells 4 min after infection (Fig. 1). However, ^{3}H-

labeled early RNA was also fully competed by RNA isolated from infected cells 18 min after infection, indicating the presence of early RNA (which could compete with the hybridization of ^{3}H-labeled early RNA) and/or the presence of RNA complementary to ^{3}H-labeled early RNA in the 18-min RNA sample. The complementary RNA could react with ^{3}H-labeled early RNA to yield duplex RNA and thus prevent the hybridization of ^{3}H-labeled early RNA to denatured gh-1 DNA.

Early RNA and/or RNA complementary to early RNA was synthesized during a 16- to 18-min period after infection, since, in hybridization competition experiments, the hybridization of RNA pulse labeled 16 to 18 min after infection was competed by early RNA (Fig. 2). When rifampin was added to the infected cell culture to inhibit *P. putida* RNA polymerase (14), early RNA and/or RNA complementary to early RNA was not transcribed during the 16- to 18-min pulse-labeling period (Fig. 3). Thus, *P. putida* RNA polymerase is most likely responsible for the synthesis of early RNA and/or RNA complementary to early RNA late during the infectious cycle. Furthermore, it is apparent that the gh-1 RNA polymerase transcribes only late RNA in vivo, because in the presence of rifampin (which inhibits the host RNA polymerase), only late RNA is synthesized.

Strand specificity of gh-1 transcription in vivo. ^{3}H-labeled early RNA synthesized in the presence of chloramphenicol hybridized almost exclusively to the L strand of gh-1 DNA

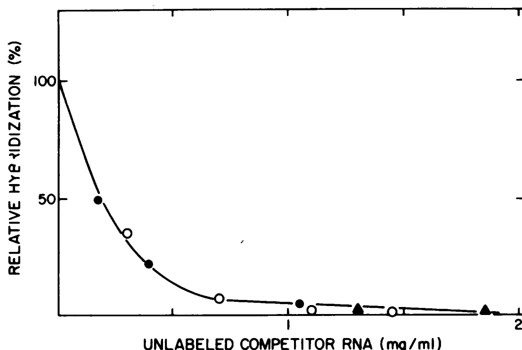

FIG. 1. *Hybridization competition with RNA pulse labeled 0 to 5 min after gh-1 infection in the presence of chloramphenicol. Labeled RNA was hybridized to denatured gh-1 DNA in the absence and in the presence of increasing amounts of competitor RNA. The competitor RNAs were RNA isolated 4 min after gh-1 infection (▲), RNA isolated 5 min after infection in the presence of chloramphenicol (●), and RNA isolated 18 min after infection (○). The procedure for pulse labeling infected cells and the conditions for hybridization competition are described in the text.*

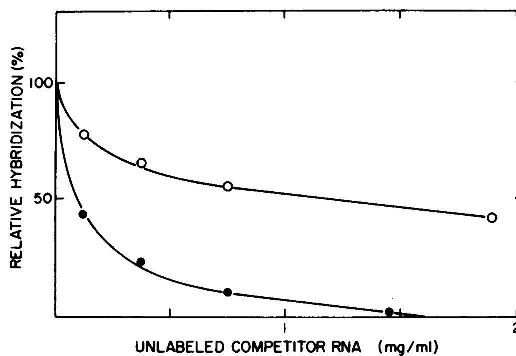

FIG. 2. *Hybridization competition with RNA pulse labeled 16 to 18 min after infection. The competitor RNAs were early RNA isolated 5 min after infection in the presence of chloramphenicol (○) and RNA isolated 18 min after infection (●).*

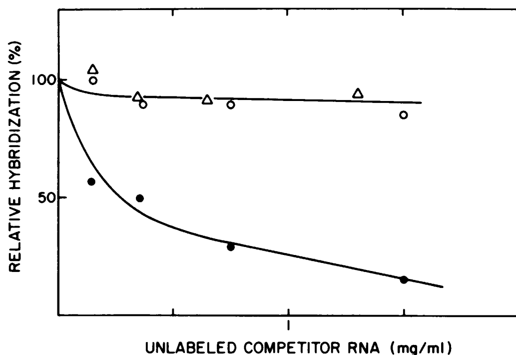

FIG. 3. *Hybridization competition with RNA pulse labeled 16 to 18 min after infection in the presence of rifampin. Rifampin (100 μg/ml) was added 5 min before the addition of ^{3}H-labeled uridine. The unlabeled competitor RNAs were E. coli RNA (△), early RNA isolated 5 min after infection in the presence of chloramphenicol (○), and RNA isolated 18 min after infection (●).*

(Table 1). Thus, *P. putida* RNA polymerase transcribes early RNA from the L strand of gh-1 DNA during the initial stage of the infectious cycle. ^{3}H-labeled RNA pulse labeled 12 to 14 and 16 to 18 min after infection hybridized to both strands of gh-1 DNA. However, when rifampin was added to the infected cell culture to inhibit the *P. putida* RNA polymerase, ^{3}H-labeled RNA pulse labeled 16 to 18 min after infection hybridized almost exclusively to the L strand of gh-1 DNA (Table 1). Thus, during the late stages of gh-1 infection, *P. putida* RNA polymerase is responsible for transcription of RNA from the H strand of gh-1 DNA and gh-1 RNA polymerase transcribes exclusively or almost exclusively the L strand of gh-1 DNA.

Synthesis of complementary RNA in vivo. The existence of complementary RNA

that under annealing conditions could form duplex RNA and become resistant to digestion by S1 nuclease was tested for during various stages of the infectious cycle. ^{3}H-labeled early RNA and RNA pulse labeled 8 to 10 min after infection did not contain complementary RNA (Fig. 4a and b). This result is consistent with the finding that these RNA species hybridize exclusively or almost exclusively to the L strand of gh-1 DNA (Table 1). In contrast, ^{3}H-labeled RNA pulse labeled 16 to 18 min after infection

TABLE 1. *Strand specificity of gh-1 RNA transcription in vivo*[a]

Time of pulse labeling of RNA	Hybridization to H strand (cpm)	Hybridization to L strand (cpm)
0–5 min (+400 μg of chloramphenicol per ml)	64	1,168
8–10 min	379	7,569
12–14 min	2,016	8,639
16–18 min	1,403	4,792
16–18 min (+100 μg of rifampin per ml)	134	3,088

[a] RNA was pulse labeled with ^{3}H-labeled uridine, extracted, and hybridized to either the H or L strand of gh-1 DNA. Chloramphenicol when present was added 5 min before infection. Rifampin when present was added 5 min before the pulse. The procedure for pulse labeling infected cells and the conditions for hybridization to separated gh-1 DNA strands are described in the text.

did contain complementary RNA (Figure 4c). As shown in Table 1, this RNA did hybridize to both the H and L strands of gh-1 DNA.

When rifampin was added to the infected cell culture, complementary RNA was not synthesized during the 16- to 18-min pulse period (Fig. 4d). As shown above, this RNA was able to hybridize to the L strand of gh-1 DNA exclusively (Table 1). Since the synthesis of complementary RNA during late stages of the infectious cycle is inhibited by the presence of rifampin, the host *P. putida* RNA polymerase is undoubtedly responsible for the synthesis of complementary RNA.

Specificity of gh-1 transcription in vitro. The in vitro transcriptional specificity of highly purified *P. putida* and gh-1 RNA polymerases was examined to determine if they are capable of duplicating in vitro the in vivo transcriptional specificity suggested for each polymerase.

P. putida RNA polymerase holoenzyme was limited to transcription of early RNA in vitro when the molar ratio of RNA polymerase to gh-1 DNA was low. For example, ^{3}H-labeled RNA synthesized in vitro when the ratio of enzyme to gh-1 DNA was 0.5 was fully competed by unlabeled early RNA (Fig. 5). It should be noted that some of the competition by unlabeled early RNA may be due to RNA duplex formation since, even at a low ratio of enzyme to gh-1 DNA, some RNA is transcribed from the H strand of gh-1 DNA in vitro (Table 2). ^{3}H-la-

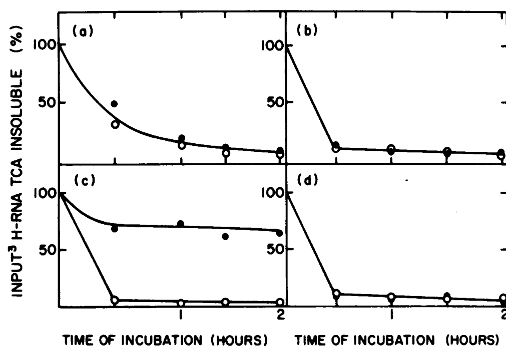

FIG. 4. *Detection of complementary RNA. ^{3}H-labeled RNA from infected cells was examined for complementary RNA as described in the text. ^{3}H-labeled RNA was denatured and incubated under annealing conditions (●) or was not incubated under annealing conditions (○), and then treated with S1 nuclease for the indicated time. ^{3}H-labeled RNAs were (a) early RNA labeled from 0 to 5 min in the presence of chloramphenicol, (b) RNA pulse labeled 8 to 10 min after infection, (c) RNA pulse labeled 16 to 18 min after infection, and (d) RNA pulse labeled 16 to 18 min after infection in the presence of rifampin (100 μg/ml) added 5 min before the pulse labeling.*

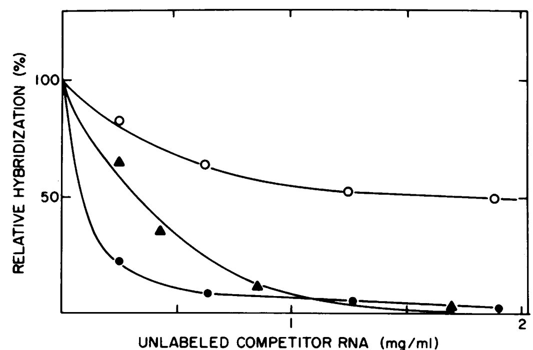

FIG. 5. *Hybridization competition with ^{3}H-labeled RNA synthesized in vitro by P. putida RNA polymerase holoenzyme and by gh-1 RNA polymerase. Hybridization competition was between ^{3}H-labeled RNA synthesized in vitro by P. putida RNA polymerase holoenzyme and early RNA from cells infected in the presence of chloramphenicol (▲), between ^{3}H-labeled RNA synthesized in vitro by gh-1 RNA polymerase and early RNA from cells infected in the presence of chloramphenicol (○), and between ^{3}H-labeled RNA synthesized in vitro by gh-1 RNA polymerase and RNA isolated from infected cells 18 min after infection (●).*

TABLE 2. *Strand specificity of gh-1 RNA transcription in vitro*[a]

RNA polymerase	Molar ratio of enzyme to gh-1 DNA	Hybridization to H strand (cpm)	Hybridization to L strand (cpm)
P. putida (holoenzyme)	50	1,731	2,616
P. putida (holoenzyme)	0.5	1,468	5,872
gh-1	50	5	2,463
gh-1	2.5	98	2,584

[a] ^{3}H-labeled RNA was synthesized in vitro with the *P. putida* holoenzyme and the gh-1 RNA polymerase at different molar ratios of enzyme to gh-1 DNA. The labeled RNA was then hybridized to the separated strands of gh-1 DNA. The procedures for in vitro RNA synthesis and hybridization are described in the text.

beled RNA synthesized when the RNA polymerase to gh-1 RNA ratio was 50 was not fully competed by unlabeled early RNA (data not shown), hybridized to both the H and L strands of gh-1 DNA (Table 2), and contained complementary RNA (data not shown).

The gh-1-induced RNA polymerase transcribed both early and late RNA in vitro (Fig. 5). This in vitro RNA hybridized to only the L strand of gh-1 DNA (Table 2) and did not contain complementary DNA (data not shown).

Thus, when the molar ratio of RNA polymerase to gh-1 DNA was low, the *P. putida* RNA polymerase was capable of transcribing only early RNA in vitro, as does the *P. putida* RNA polymerase in vivo during the initial stage of phage infection. However, transcription by the *P. putida* RNA polymerase was not completely limited to the L strand in vitro (Table 2), and it is not known if the *P. putida* RNA polymerase utilized the correct early promoters in vitro. At high molar ratios of host RNA polymerase to gh-1 DNA, complementary RNA was made in vitro, whereas at late stages of phage infection complementary RNA is made by the host enzyme in vivo.

The gh-1 RNA polymerase only partially duplicated in vitro the specificity it exhibited in vivo. The gh-1 RNA polymerase was capable of transcribing late RNA from the L strand of gh-1 DNA, but it also transcribed early RNA from the L strand.

DISCUSSION

The following model for gh-1 transcription is suggested from the results presented in this paper. During the initial stage of gh-1 infection *P. putida* RNA polymerase transcribes early RNA from the L strand of gh-1 DNA. One of the early RNA species presumably codes for gh-1 RNA polymerase which then transcribes late RNA from the L strand of gh-1 DNA. During the late stage of gh-1 infection, *P. putida* RNA polymerase transcribes RNA from both the L and the H strand of gh-1 DNA and synthesizes complementary RNA.

Several unique features of gh-1 transcription are apparent. First, the transcriptional activity of the *P. putida* RNA polymerase is not shut off during gh-1 infection but continues to transcribe gh-1 DNA throughout the infectious cycle. In contrast, during T7 infection, the *E. coli* RNA polymerase is inhibited early in the infectious cycle (4, 5). Second, complementary RNA is transcribed late in the gh-1 infectious cycle. A small amount of complementary RNA has been detected during T4 (11, 18) and λ (3) phage infection of *E. coli* and in vaccinia virus-infected chicken cells (7). A relatively large amount of complementary RNA has been detected only in HeLa cell mitochondria (1, 26).

Since *P. putida* RNA polymerase is not shut off during gh-1 phage infection, it can be assumed that either (i) gh-1 does not have a mechanism for shutting off the host RNA polymerase or (ii) the host RNA polymerase is required throughout the gh-1 infectious cycle. The first possibility is more likely because when rifampin was added to the infected cell culture (as early as 12 min postinfection) to inhibit the *P. putida* RNA polymerase, phage production was not significantly affected (data not shown), indicating that the host RNA polymerase is not required late in infection. It is possible, however, that under different conditions of infection or in a different host cell, phage gh-1 may either be able to shut off the host RNA polymerase or may require the presence of the host RNA polymerase throughout the infectious cycle. It is also possible that gh-1 is a mutant of a yet undiscovered parental phage which is capable of host RNA polymerase shutoff.

Complementary RNA is undoubtedly synthesized by the *P. putida* RNA polymerase late in the infectious cycle because, when rifampin was added to the infected cell culture, no complementary RNA was made. There are at least three possible explanations for the synthesis of complementary RNA late in the infectious cycle. (i) The *P. putida* RNA polymerase may be modified late in infection so that it utilizes promoters on the H strand of gh-1 DNA and synthesizes complementary RNA. (ii) The gh-1 DNA template may be altered structurally late in infection, perhaps as a result of phage particle maturation, so that the *P. putida* RNA polymerase can transcribe the H strand of gh-1 DNA. (iii) The molar ratio of *P. putida* RNA poly-

merase to gh-1 DNA could increase late in infection due to the degradation of host DNA and release of host RNA polymerase molecules from the degraded host genome. The excess *P. putida* RNA polymerase would then transcribe RNA from weak promoters on the H strand of gh-1 DNA not utilized when enzyme is not in excess. The results in this paper indicate that *P. putida* RNA polymerase does transcribe RNA from the H strand of gh-1 DNA in vitro when enzyme is in excess.

ACKNOWLEDGMENTS

I wish to thank John A. Boezi for his helpful discussions and help with the preparation of the manusccipt.

This work was supported in part by Public Health Service predoctoral trainee grant ST01GM 1091 from the National Institutes of Health.

LITERATURE CITED

1. **Aloni, Y., and G. Attardi.** 1971. Symmetrical *in vivo* transcription of mitochondrial DNA in HeLa cells. Proc. Natl. Acad. Sci. U.S.A. **68**:1757-1761.
2. **Bøvre, K., and W. Szybalski.** 1971. Multistep DNA-RNA hybridization techniques. Methods Enzymol. **21**: 350-383.
3. **Bøvre, K., and W. Szybalski.** 1969. Patterns of convergent and overlapping transcription within the b2 region of coliphage λ. Virology **38**:614-626.
4. **Brunovskis, I., and W. C. Summers.** 1971. The process of infection with coliphage T7. V. Shutoff of host RNA synthesis by an early phage function. Virology **45**:224-231.
5. **Brunovskis, I., and W. C. Summers.** 1972. The process of infection with coliphage T7. VI. A phage gene controlling shutoff of host RNA synthesis. Virology **50**: 322-327.
6. **Chamberlin, M., J. McGrath, and L. Waskell.** 1970. New RNA polymerase from *Escherichia coli* infected with bacteriophage T7. Nature (London) **228**:227-231.
7. **Colby, C., and P. H. Duesberg.** 1969. Double-stranded RNA in vaccinia virus infected cells. Nature (London) **222**:940-944.
8. **Davis, R. W., and R. W. Hyman.** 1970. Physical locations of the *in vitro* RNA initiation site and termination sites of T7 DNA. Cold Spring Harbor Symp. Quant. Biol. **35**:269-281.
9. **Dunn, J. J., and F. W. Studier.** 1973. T7 early RNAs are generated by site-specific cleavages. Proc. Natl. Acad. Sci. U.S.A. **70**:1559-1563.
10. **Dunn, J. J., and F. W. Studier.** 1973. T7 early RNAs and *Escherichia coli* ribosomal RNAs are cut from large precursor RNAs *in vivo* by ribonuclease III. Proc. Natl. Acad. Sci. U.S.A. **70**:3296-3300.
11. **Geiduschek, E. P., and O. Grau.** 1970. T4 anti-messenger, p. 190-203. *In* L. Silvestri (ed.), RNA polymerase and transcription. Lepetit Colloquim on Biology and Medicine (first), North-Holland Publishing Co., Amsterdam.
12. **Hercules, K., M. Schweiger, and W. Sauerbier.** 1974. Cleavage by RNase III converts T3 and T7 early precursor RNA into translatable message. Proc. Natl. Acad. Sci. U.S.A. **71**:840-844.
13. **Hyman, R. W.** 1971. Physical mapping of T7 messenger RNA. J. Mol. Biol. **61**:369-376.
14. **Johnson, J. C., M. DeBacker, and J. A. Boezi.** 1971. Deoxyribonucleic acid-dependent ribonucleic acid polymerase of *Pseudomonas putida*. J. Biol. Chem. **246**: 1222-1232.
15. **Kindler, P., T. U. Keil, and P. H. Hofschneider.** 1973. Isolation and characterization of a ribonuclease III deficient mutant of *Escherichia coli*. Mol. Gen. Genet. **126**:53-69.
16. **Lee, L. F., and J. A. Boezi.** 1966. Characterization of bacteriophage gh-1 for *Pseudomonas putida*. J. Bacteriol. **92**:1821-1827.
17. **Millette, R. L., C. D. Trotter, P. Herrlich and M. Schweiger.** 1970. *In vitro* synthesis, termination, and release of active messenger RNA. Cold Spring Harbor Symp. Quant. Biol. **35**:135-142.
18. **Notani, G. W.** 1973. Regulation of bacteriophage T4 gene expression. J. Mol. Biol. **73**:231-249.
19. **Nygaard, A. P., and B. D. Hall.** 1964. Formation and properties of RNA-DNA complexes. J. Mol. Biol. **9**: 125-142.
20. **Siegal, R. B., and W. C. Summers.** 1970. The process of infection with coliphage T7. III. Control of phage-specific RNA synthesis *in vivo* by an early phage gene. J. Mol. Biol. **49**:115-123.
21. **Summers, W. C., and R. B. Siegel.** 1970. Regulation of coliphage T7 RNA metabolism *in vivo* and *in vitro*. Cold Spring Harbor Symp. Quant. Biol. **35**:253-257.
22. **Szybalski, W., H. Kubinski, Z. Hradecna, and W. C. Summers.** 1971. Analytical and preparative separation of the complementary DNA strands. Methods Enzymol. **21**:383-413.
23. **Thomas, C. A., and J. Abelson.** 1966. The isolation and characterization of DNA from bacteriophage, p. 553-561. *In* G. L. Cantoni and D. R. Davies (ed.), Procedures in nucleic acid research. Harper and Row, New York.
24. **Towle, H. C., J. F. Jolly, and J. A. Boezi.** 1975. Purification and characterization of bacteriophage gh-1-induced deoxyribonucleic acid-dependent ribonucleic acid polymerase from *Pseudomonas putida*. J. Biol. Chem. **250**:1723-1733.
25. **Vogt, V. M.** 1973. Purification and further properties of single-strand-specific nuclease from *Aspergillus oryzae*. Eur. J. Biochem. **33**:192-200.
26. **Young, P. G., and G. Attardi.** 1975. Characterization of double-stranded RNA from HeLa cell mitochondria. Biochem. Biophys. Res. Commun. **65**:1201-1207.
27. **Zillig, W., H. Fujiki, W. Blum, D. Janekovic, M. Schweiger, H.-J. Rahmsdorf, H. Ponta, and M. Hirsch-Kauffmann.** 1975. *In vivo* and *in vitro* phosphorylation of DNA-dependent RNA polymerase of *Escherichia coli* by bacteriophage-T7-induced protein kinase. Proc. Natl. Acad. Sci. U.S.A. **72**:2506-2510.